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Optimization of Liquid Culture Conditions of *Coprinopsis cinerea* as Natural Source of Bioactive Compounds

Rich Milton R. Dulay*, Ethel Mae G. Cardona, Sofronio P. Kalaw and Renato G. Reyes

Center for Tropical Mushroom Research and Development, Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija 3120 Philippines

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ABSTRACT

In the Philippines, mushrooms are cultivated using different formulated cellulosic substrates and the liquid cultivation of mycelial biomass as source of natural bioactive compounds is less intensively practiced. Herein, this present work demonstrated the optimization of liquid culture conditions for the mycelial biomass production of C. cinerea intended for the elucidation of active chemical components with important biological activities. The maximum yield of mycelial biomass of C. cinerea was attained when grown in potato sucrose broth with pH 7.5 and incubated in room temperature (30°C), alternating light and dark, and agitated at 70 rpm condition. Mycelia produced in static condition had higher radical scavenging activity (91.43%) than the culture spent. In contrast, culture spent had higher total phenolic content (210 mg AAE/g sample) than its mycelia. However, in agitated liquid culture, culture spent showed higher radical scavenging activity (77.14%) and total phenolic content (235 mg AAE/g sample) than its corresponding mycelia, which is a strong evident of the oozing of active metabolites from mycelia to the culture spent. Mycochemical analysis revealed that mycelial biomasses produced in both static and agitated condition contained varying amounts of saponins, flavonoids, cardiac glycosides, alkaloids and terpenoids, which are known for their numerous biological activities. Therefore, the established optimum liquid culture of C. cinerea is useful not only for mycelial biomass production but most importantly as a natural resource of bioactive metabolites. Evaluation of other functional bioactivities of the liquid culture of C. cinerea using the optimum culture conditions is currently under investigation.

Keywords: *C. cinerea*, liquid culture, indigenous media, antioxidant, mycochemicals.

INTRODUCTION

Coprinopsis cinerea of Family Psathyrellaceae is a leaf-litter decomposing basidiomycetous fungus that usually found growing on the pile of decaying rice straw. This mushroom is typically has a stipe length of 70 to130 mm and grayish tone pileus which turns to inky cap when matures, indicating autolysis for spore dispersal. It is an edible species and considered a native mushroom by Filipinos which is collected from the yard and field and prepared as main ingredient of some native delicacies. Several investigation about *C. cinerea* have focused on its used as representative specimen to illustrate gene segregations, cytological, biochemical, and morphological aspects of the morphogenesis and developmental processes in the homobasidiomycetous fungi [1, 2]. In addition, *C. cinerea* is also a model in meiosis study due to its synchronous meiotic development and prolonged prophase [3].

Optimization of culture condition is an important strategy to develop a successful production technology of the desired mushrooms. In the Philippines, mushrooms are cultured in the different formulated substrates such as agro-industrial wastes and other cellulosic residues. The submerged cultivation of mycelial biomass for bioactive metabolites production in liquid media is less intensively practiced. This cultivation technique is more advantageous because mushroom can produce high biological efficiency in a short incubation period with less chance of contamination, and most importantly, it is useful and easily accessible source of extractable natural compounds with

various biological activities. In the review conducted by Elisashvili [4], the fruiting bodies, culture mycelium, and culture broth of mushrooms contain secondary metabolites including polysaccharides, proteins and their complexes, phenolic compounds, polyketides, triterpenoids, steroids, alkaloids, nucleotides, etc., which have been associated to their cholesterol-lowering, anti-diabetic, antioxidant, antitumor, immunomodulating, antimicrobial, and antiviral properties.

Recently, the liquid culture conditions (culture broth, pH, temperature and shaking condition) of four Philippine wild edible mushrooms namely; *Ganoderma lucidum*, *Pleurotus cystidiosus*, *Volvariella volvacea*, and *Schizophyllum commune* were optimized for mycelial biomass production and bioactive lipids elucidation [5]. The maximum mycelial biomasses of *G. lucidum*, *P. cystidiosus*, *V. volvacea* and *S. commune* favourably produced in Sabouraud dextrose broth at pH 7, 7, 6 and 8, respectively, when incubated at 28°C and 30°C. Moreover, the influence of the different indigenous culture broth on the antioxidant and total phenolic contents of *Lentinus tigrinus*, *Lentinus sajor-caju*, *S. commune* and *V. volvacea* was also investigated and it was found out that the properties of these mushrooms varied when cultured in the different indigenous culture broth [6, 7].

To the best of our knowledge, very little has been written so far on the optimization of liquid culture conditions for mycelial biomass production and there are no comprehensive data on the antioxidant activity and mycochemical composition of the Philippine local strain of wild *C. cinerea*. Herein, we optimized the liquid culture conditions of this mushroom as influenced by the different indigenous culture broth, pH, temperature, illumination, and shaking condition for the production of mycelia biomass as natural source of bioactive compounds.

MATERIALS AND METHODS

Source of Mushroom

Pure culture of *C. cinerea* was obtained from the culture collection of Center for Tropical Mushroom Research and Development (CTMRD), Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija.

Preparation of Culture Inoculant

An agar block from the pure culture *C. cinerea* was aseptically inoculated into a prepared potato sucrose agar (PSG) plates. Culture plates were incubated at room temperature to allow mycelia growth. After 7 days of incubation, mycelial discs were prepared using a flame sterile 10 mm-diameter cork borer. Mycelial discs were served as culture inoculant in the evaluation of the optimum liquid culture conditions.

Evaluation of the Optimum Liquid Culture Conditions

The effect of different broth culture media namely; coconut water from mature coconut (*Cocos nucifera*), rice bran D1 (class A) broth (50g of *Oryza sativa*/L of water), local yellow corn grit broth (50g of *Zea mays*/L of water) and potato sucrose broth (250g of *Solanum tuberosum*/L of water + 10g of white table sugar) on mycelia growth of the *C. cinerea* in liquid culture condition was evaluated. Broth media (100 ml) were dispensed into autoclavable conteiner, sterilized in an autoclave at 121°C, 15 psi for 30 min, aseptically inoculated with mycelia discs, and incubated at 30°C to allow fungal growth. After 10 days of incubation, the mycelia were harvested, air-dried, and weighed to determine the most favourable broth medium for efficient mycelial biomass production of *C. cinerea*.

The best medium from the preceding media evaluation was adjusted to varying pH levels (5.0 - 8.0) with 0.5 intervals using 0.1 M NaOH and HCl. One hundred ml of the each pH broth was dispensed in a flask, sterilized, inoculated and incubated for 10 days. Mycelial biomasses were air-dried. Mycelial discs were aseptically inoculated into flask containing 100 ml of the best medium at optimum pH level and incubated at different temperature conditions (9, 23, and 30°C) to determine the optimum temperature requirement of the mushroom. Culture at the optimum medium, pH, and temperature was evaluated to different illumination conditions (dark, lighted, and alternate-12 hrs dark and 12 hrs light) to determine the optimum illumination requirement of *C. cinerea.* Finally, the optimum medium, pH, temperature, and illumination previously determined were employed in optimizing shaking conditions (static and agitated at 70 rpm). Experiments were carried out in triplicate. The weight of the air-dried mycelial biomass was determined and data were presented as the mean of three replicates.

Mass Production of Mycelial Biomass

Mass production of mushrooms mycelia was carried out by inoculating mycelia discs in autoclavable container containing 100 ml of the best medium at optimum pH and incubated in the required temperature, illumination, and both static and shaking conditions for 10 days. The mycelial biomasses were harvested and the culture spent were collected and subjected for antioxidant and mycohemical content analyses.

DPPH Radical Scavenging Activity

Ethyl acetate (10 ml) was added into each sample of mycelia and spent to extract the antioxidant compounds. The ethyl acetate soluble portion was concentrated under reduced pressure and the concentrates were dissolved in ethanol. The free radical scavenging activity of the samples was estimated using the stable 2,2'-diphenyl1-1picrylhydrazyl (DPPH) radical following the standard method of Shimada *et al*. [8] (with modifications. A 100 µl of test sample in ethanol was added with 5 µl DPPH solution (5 mg DPPH powder in 2 ml of ethanol) in 96-well microtitter plates. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517 nm. The inhibition of DPPH free radicals was calculated. Triplicate test was done per sample.

Estimation of Total Phenolic

The total phenolic content was estimated using Folin-Ciocalteu method of Slinkard and Singleton [9] with modifications. Sample solution (50 µl) was mixed 500 µl of 10% Folin-Ciocalteu reagent (Folin:Methanol, 1:1, v/v). After 2 min, 50 µl of 7.5% saturated was added and kept in the dark for 1h before absorbance was taken at 765 nm. A calibration curve was obtained using various concentrations of ascorbic acid. The total phenolic content of the sample was expressed as mg of ascorbic acid equivalents (AAEs) per gram of sample. Triplicate test was done per sample.

Mycochemical Analyses

The chemical screening of the aqueous extracts of mycelia were carried out following the procedures described by Sofowora [10] and Harborne [11]. Three replicates were laid out for each test parameter. Results were compared with distilled water as control and determined based on the color/intensity of the reaction [12].

Statistical Analysis

The data on the optimization study were analyzed using One Way Analyses of Variance (ANOVA). Significant difference of means were analyzed using Tukey's Honestly Significant Differences Test at 5% level of significance in SPSS Version 17. 00.

RESULTS AND DISCUSSION

Influence of Liquid Culture Media

Mushroom fruiting body cultivation is a long-term process. Therefore, cultivation of mycelial biomass in a liquid culture media is being introduced because of the shorter incubation period having biomass yield in just few days. However, the chemical and physical factors (including nutritive media, pH level, temperature, illumination, and agitation) for growth of mycelia in liquid culture must take into consideration. In this study, the mycelial biomass of *C. cinerea* in different indigenous broth media was evaluated (Table 1). Potato sucrose broth significantly produced the highest biomass yield of 203.70 mg, followed by rice bran broth with 143.23 mg. However, corn grit broth produced the lowest biomass yield of 40.93 mg. These results indicate that potato sucrose broth favors the luxuriant growth of mycelial biomass of *C. cinerea*. This conforms with optimum broth medium for the mycelial biomass production of *Calocybe indica* and *Panaeolus antillarium* [13, 14]. Moreover, the maximum biomass production of *Pleorotus florida*, *P. sajor-caju*, *P. citrinopileatus*, *P. ostreatus*, and *P. opuntiae* was attained in potato broth [15].

The pH of the media has a remarkable influence on the morphology and growth of mushroom mycelia. The influence of varying pH levels of potato sucrose broth on the mycelial biomass of *C. cinerea* was determined and the results are presented in Table 1. It can be seen that pH 7.5 produced the highest biomass yield of 377.56 mg, thus, it is the optimum pH for the efficient mycelial biomass production of *C. cinerea*. However, no mycelial growth was noted in the media at pH 5, pH 5.5, and pH 6, which suggest that acidic medium has inhibitory effect in *C. cinerea* mycelial growth.

Table 1. Influence of nutritional and physical factors on the mycelial biomass production of *C. cinerea* in liquid culture

Nutritional / Physical Factors	Yield of Mycelial Biomass (mg)
Broth Media	
Coconut water	42.93±29.73[c]
Rice bran broth	143.23±36.79[b]
Corn grit broth	40.93±22.37[c]
Potato sucrose broth	203.70±83.16[a]
pH	
5.0	0.00±0.00[d]
5.5	0.00±0.00[d]
6.0	0.00±0.00[d]
6.5	237.43±28.86[c]
7.0	184.36±08.21[c]
7.5	337.56±49.75[a]
8.0	316.56±37.91[b]
Temperature	
Refrigerated (9°C)	0.00±0.00
Air-conditioned (23°C)	153.33±129.2[b]
Room temperature (30°C)	400.16±11.15[a]
Illumination	
Dark	225.53±21.46[b]
Lighted	206.33±78.20[b]
Alternate	432.43±23.33[a]
Shaking Condition	
Static	335.56±08.15[b]
Agitated (70 rpm)	351.93±34.95[a]

Values are expressed as Mean ±SD of triplicates. In each factor, values with the same letter superscript are not significantly different at P ≤ 0.05 using Tukey's HSD.

Effect of Physical Factors

The effect of the three important physical factors (temperature, illumination and shaking conditions) in the mycelial biomass production of *C. cinerea* was also evaluated in this study. Three different temperature conditions (refrigerated 9°C, air-conditioned 23°C and room temperature 30°C) were evaluated and the results are also presented in Table 1. Apparently, the highest yield of mycelial biomass was significantly recorded to those incubated at room temperature with 400.16 mg, followed by air-conditioned having 153.33 mg. This strongly suggests that *C. cinerea* is a tropical species of mushroom. In contrast, no mycelial growth was observed to those incubated in refrigerated condition. This result agrees with the study of Garraway and Evans [16] who reported that metabolic activities of fungi are always reduced at extremely low temperature. The reduction of the mycelial growth at very low temperature may be due to the reducing metabolic activities of the mushroom which allow the absorption of essential nutrients needed for its growth.

Another physical factor which is vital for the mycelial growth is illumination condition. Alternating light and dark condition produced the highest biomass yield of 432.4 mg, indicating the optimum illumination condition for the mycelial growth of *C. cinerea*. Light is needed to synchronize development within the day/night rhythm. If kept in the dark condition, they transform into sclerotia for survival under adverse environmental conditions. When light is provided, primary hyphal knots convert into the more compact secondary hyphal knots in which tissue differentiation occurs. This may be also in relation with mycelial growth since mushroom fruiting body was derived from mycelia [17].

Finally, the influence of static and agitated condition (70 rpm) was also evaluated. It can be noticed that agitated condition favoured the highest yield of 351.93 mg. These results suggest that agitated condition is required for the mycelial growth of *C. cinerea*. However, the optimum rpm of agitation should take into consideration in the future study. In the study conducted by Upadhyay and Fritsche [18], the maximum mycelial biomass was observed under shaking condition in case of *Pleurotus citrinopileus, P. florida,* and *P. sapidus* while *P. sajor-caju, C. comatus, K. mutabilis, L. edodes* and *T. rutilans* produced the maximum biomasss under static condition. Furthermore, it was also reported that there is an increase in yield of mycelium of *G. lucidum* when the shaking frequency was from 50 rpm to 100 rpm, which implies a better oxygen transfer in broth medium which is needed for the growth of mycelia [19].

Radical Scavenging Activity

Antioxidants are substances that neutralize free radicals or their actions [20]. Every cell has adequate protective mechanisms against any harmful effects of free radicals; antioxidants are buffering systems in every cell [21]. Moreover, antioxidants reduce the effect of cancers and cardiovascular diseases [22], neurodegenerative diseases [23], diabetes [24] and many more diseases. The radical scavenging activity of the mycelial biomass and culture spent of *C. cinerea* in static and agitated liquid culture conditions was studied (Table 2). Mycelia from static

condition registered higher radical scavenging activity (91. 43%) than the mycelia from agitated condition (61.90%). These results are in accordance with the study conducted by Arora and Chandra [25], wherein static culture of two isolates of *Aspergillus* spp. gave better radical scavenging activity when compared to shake flask culture. However, in terms of their culture spent, agitated condition had higher scavenging activity (77.14%) compared to those in static condition (73.33%). This clearly indicates the oozing of the bioactive metabolites from the mycelia to their culture spent. Agitation breaks the mycelial cellwall and diffuses the active metabolites to their culture spent.

The radical scavenging activity of the mushroom species is attributed to the presence of the different mycochemicals, specifically the phenolic compounds such as alkaloids, flavonoids, and glycosides [26, 27, 28]. Aside from this work, other previous studies have also reported the radical scavenging activity of several mushrooms. Some of these include *Ganoderma lucidum, Grifola umbellata, Coriolus versicolor, Tricholoma lobayense, Volvariella volvacea, Tremella fuciformis, Pleurotus florida*, and *Panaeolus antillarium* [14, 29, 30].

Table 2. Radical scavenging activity and total phenolics of mycelia and culture spent of *C. cinerea* in static and agitated liquid culture

Condition	Sample	Radical Scavenging Activity (%)	Total Phenolic Content (mg AAE/g sample)
Static	Mycelia	91.43	156.00
	Spent	73.33	210.00
Agitated	Mycelia	61.90	78.00
	Spent	77.14	235.00
Control	Cathechin	98.10	-

Total Phenolic Content

Phenolics are considered antioxidants because of their ability to chelate metals, inhibit lipoxygenase and scavenge free radicals [31]. The total phenolic content of the samples was determined spectrophotometrically using the Folin-Ciocalteu method. The results of total phenolics analysis of mycelial biomass and culture spent produced in static and agitated liquid culture conditions are also presented in Table 2. Interestingly, culture spent of agitated liquid culture contained the highest phenolics (235 mg AAE/g sample) while its mycelia had the lowest phenolic content (78 mg AAE/g sample), among the samples analyzed. On the other hand, the mycelia produced in static culture had 156 mg AAE/g sample, which is lower when compared to its culture spent with 210 mg AAE/g sample. These results also dictate that agitation would allow the diffusion of bioactive metabolites to the culture spent. The total phenolic contents of *C. cinerea* obtained in the present study are far higher compared to *Coprinus comatus* (17.82 mg AAE/g sample), *Pleurotus cystidiosus* (3.41 mg AAE/g sample), and *Panaeolus antillarium* (25.11 mg AAE/g sample) [14, 32]. Thus, phenolic content of mushrooms vary depending on the species and strains.

Mycochemical Screening of C. cinerea

Mycochemicals are compounds present in fungi which are responsible in different metabolic functions. The mycochemicals including tannins, saponins, flavonoids, terpenoids, cardiac glycosides, and alkaloids present in the mycelial biomass of *C. cinerea* were screened in the present study. The mycochemical compositions of extracts of the air-dried mycelial biomass of *C. cinerea* in static and agitated liquid culture are shown in Table 3. Out of six mycochemical screened, five mycochemicals namely; saponins, flavonoids, terpenoids, cardiac glycosides, and alkaloids were found present in the mycelia produced in both static and agitated conditions. However, tannins were not detected in both samples. Saponins and cardiac glycosides of mycelia in static condition were detected in appreciable amounts but present in trace amounts in agitated condition. Flavonoids, on the other hand, were detected in appreciable amount in agitated produced-mycelia but found in trace amount in static produced-mycelia. Terpenoids were present in appreciable amount in the mycelia of both conditions. These results strongly indicate that the amount of mycochemical compositions of mycelia of *C. cinerea* may vary depending on the physical conditions of the liquid culture.

Table 3. Mycochemical compositions of mycelial biomass of *C. cinerea* in static and agitated liquid culture

Mycochemicals	Mycelial Biomass of *C. cinerea*	
	Static	Agitated
Tannins	0	0
Saponins	++	+
Flavonoids	+	++
Terpenoids	++	++
Cardiac glycosides	+	+
Alkaloids	++	+

In column, (+) present in trace amounts, (++) present in appreciable amount, (0) absent

Mycochemicals are active compounds responsible to many biological activities and pharmacological properties. Saponins are potent antioxidants that neutralize free radicals to prevent disease and stimulate the production of

antibodies, which help in fighting bacterial and fungal infections [33]. Other pharmacologic effects of saponins include haemolytic, anti-inflammatory, antifungal, anti-bacterial, anti-viral, ichthyotoxic, cytostatic and antieoplastic activities [34]. Flavonoids are considered as natural sources of phenolic antioxidants [35]. Some flavonoids have been reported to possess a variety of biological activities, including antiallergic, anti-inflammatory, antiviral, antiproliferative, and anticarcinogenic activities [36]. Moreover, since flavonoids are good source of antioxidants, they can help in prevention of human diseases such as cancer and cardiovascular diseases, and some pathological disorders of gastric and duodenal ulcers, allergies, vascular fragility, and viral and bacterial infections [37].

Terpenes, on the other hand, are reported to have a unique antioxidant activity in their interaction with free radicals [38]. In some studies, some terpenoids suppressed the growth of diverse tumor cell lines via initiation of apoptosis [39]. Carotenoid terpenes protect against uterine, prostate, breast, colorectal and lung cancers. They may also protect against risk of digestive tract cancer [40]. Some terpenoids exhibit anti-inflammatory), anti-neoplastic, anti-pyretic and immune-modulating activity [41]. In the review made by Prassas and Diamandis [42], cardiac glycosides have been a cornerstone of the treatment of heart diseases and are potential anticarcinogens. Cardiac glycosides are also used to treat cystic fibrosis [43], and considered as the most potent neuroprotective for ischaemic stroke [44]. However, alkaloids have been known for their therapeutic capabilities including anticancer, antiasthma, and antimalaria [45]. Some alkaloids have analgesic, antihyperglycemic and antibacterial properties [46, 47, 48].

In conclusion, potato sucrose broth with pH 7.5 incubated in room temperature (30°C), alternating light and dark, and agitated at 70 rpm were the optimum liquid culture conditions for the mycelial biomass production of *C. cinerea*. In static condition, mycelia had higher radical scavenging activity while its culture spent had higher total phenolic content than its corresponding mycelia. In contrast, the maximum radical scavenging activity and total phenolic content was significantly recorded in the culture spent of agitated liquid culture, indicating the oozing the active metabolites from the mycelia to the culture spent due to agitation. In terms of mycochemical screening, both mycelial biomasses produced in static and agitated condition contained varying amounts of saponins, flavonoids, cardiac glycosides, alkaloids and terpenoids. These important results strongly indicate the great potential of *C. cinerea* in nutraceutical and pharmacological purposes. The evaluation of other functional bioactivities of the liquid culture of *C. cinerea* using the established optimum culture conditions is currently under investigation.

REFERENCES

[1] Moore D, Pukkila PJ. *Journal of Biological Education*, **1985**, 19(1):31–40.
[2] Wälti MA, Villalba C, Buser RM, Grünler A, Aebi M, Künzler M. *Eukaryotic Cell*, **2006**, 5(4):732–744.
[3] Burns C, Stajich JE, Rechtsteiner A, Casselton L, Hanlon SE, Wilke SK, Pukkila PJ. *PLoS Genet*, **2010**, 6.9, e1001135.
[4] Elisashvili V. *International Journal of Medicinal Mushrooms*, **2012**, 14(3):211–239.
[5] Dulay RMR, Ray K, Hou CT. *Biocatalysis and Agricultural Biotechnology*, **2015**, 4:409–415.
[6] Dulay RMR, Flores KS, Tiniola RC, Marquez DHH, Dela Cruz AG, Kalaw SP, Reyes RG. *Mycosphere*, **2015**, 6(6):659–666.
[7] Dulay RMR, Vicente JJA, Dela Cruz AG, Gagarin JM, Fernando W, Kalaw SP, Reyes RG. *Mycosphere*, **2016**, 7(2):131–138.
[8] Shimada K, Fujikawa K, Yahara K, Nakamura T. *J Agri Food Chem*.**1992**, 40:945–948.
[9] Slinkard K, Singleton VL. *Am. J. Enol. Vitic.*, **1977**, 28:49–55.
[10] Sofowara A. Medicinal plants and traditional medicine in Africa. Spectrum Books. Ltd, Ibadan, Nigeria. **1993**, 289.
[11] Harborne JB. Phytochemical methods, London. Chapman and Hall, Ltd. **1973**, 188.
[12] Guevara BQ. A Guidebook to Plant Screening: Phytochemical and Biological. Revised Edn. UST Publishing House, Manila, Philippines. **2005**, 156.
[13] Phutela UG, Phutela RP. *Int J Adv Life Sci*., **2012**, 2:8–16.
[14] Dulay RMR, Cabalar AC, De Roxas MJB, Concepcion JMP, Cruz NE, Esmeralda M, Jimenez N, Aguilar JC, De Guzman EJ, Santiago JQ, Samoy JR, Bustillos RG, Kalaw SP, Reyes RG. *Current Research in Environmental & Applied Mycology*, **2015**, 5(1):52–59.
[15] Suharban M, Nair MC. *Proceedings of National Symposium on Mushrooms*. **1991**, 139–140.
[16] Garraway MO, Evans RC. Fungal Nutrition and Physiology. New York. Wiley, **1984**, 71-292.
[17] Rühl M, Majcherczyk A, Kües U. *Antonie van Leeuwenhoek*, **2013**, 103(5), 1029–1039.
[18] Upadhyay RC, Fritsche W. *Advances in Mushroom Biology and Production (Rai, Dhar and Verma eds.) M. S. I.*, **1997**, 281–290.
[19] Yang FC, Liau CB. *Process Biochemistry*, **1998**, 33(5):547–553.
[20] Sies H. *Angewandte Chemie International Edition in English*, **1986**, 25(12):1058–1071.
[21] Devasagayam TPA, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, Lele RD. *Japi*, **2004**, 52:794–804.

[22] Chopra M, Mcloone U, O'neill M, Williams M, Thurnham DJ. *Special Publications of the Royal Society of Chemistry*, **1996**, 181:150–155.
[23] Yoshikawa T, Toyokuni S, Yamamoto Y, Naito Y. Free radicals in Chemistry, Biology and Medicine. OICA International (UK). **2000**, 580.
[24] Lipinski B. *Journal of Diabetes and its Complications*, **2001**, 15(4):203–210.
[25] Arora DS, Chandra P. *Brazilian Journal of Microbiology*, **2010**, 41(3):765–777.
[26] Pietta PG. *Journal of Natural Products*, **2000**, 63(7):1035–1042.
[27] Herraiz T, Galisteo J. *Free Radical Research*, **2002**, 36(8):923–928.
[28] Zhang X, Thuong PT, Min BS, Ngoc TM, Hung TM, Lee IS, Na M, Seong YH, Song KS, Bae K. *Journal of Natural Products*, **2006**, 69(9):1370–1373.
[29] Liu F, Ooi VEC, Chang ST. *Life Sciences*, **1997**, 60(10):763–771.
[30] Menaga D, Rajakumar S, Ayyasamy PM. *Int J Pharm Pharm Sci*, **2013**, *5*(4):601–606.
[31] Martínez-Valverde I, Periago MJ, Ros G. *Archivos Latinoamericanos de Nutricion*, **2000**, 50(1):5–18.
[32] Kalaw SP, Albinto RF. *Mycosphere*, **2014**, 5(5):646–655.
[33] Imaga NOA, Adenekan SO, Fakanye TJ, Olaniyan FK, Alakaloko AA. *Unilag Journal of Medicine, Science and Technology*, **2013**, 1(1):1–8.
[34] Caulier G, Van Dyck S, Gerbaux P, Eeckhaut I, Flammang P. *SPC Beche-de-Mer Inf. Bull*., **2011**, 31:48–54.
[35] Yao LH, Jiang YM, Shi J, Tomas-Barberan FA, Datta N, Singanusong R, Chen SS. *Plant Foods for Human Nutrition*, **2004**, 59(3):113–122.
[36] Ren W, Qiao Z, Wang H, Zhu L, Zhang L. *Medicinal Research Reviews*, **2003**, 23(4), 519–534.
[37] Zand RSR, Jenkins DJ, Diamandis EP. *Journal of Chromatography B*, **2002**, 777(1):219–232.
[38] Dillard CJ, German JB. *Journal of the Science of Food and Agriculture*, **2000**, 80(12):1744–1756.
[39] Mo H, Elson CE. *The Journal of Nutrition*, **1999**, 129(4):804–813.
[40] Franceschi S, Bidoli E, Vecchia CL, Talamini R, D'avanzo B, Negri E. *International Journal of Cancer*, **1994**, 59(2):181–184.
[41] Hertog MG, Feskens EJ, Kromhout D, Hollman PCH, Katan MB. *The Lancet*, **1993**, 342(8878):1007–1011.
[42] Prassas I, Diamandis EP. *Nature Reviews Drug Discovery*, **2008**, 7(11):926–935.
[43] Manna SK, Sreenivasan Y, Sarkar A. *Journal of Cellular Physiology*, **2006**, 207(1):195–207.
[44] Wang JK, Portbury S, Thomas MB, Barney S, Ricca DJ, Morris DL, Lo DC. *Proceedings of the National Academy of Sciences*, **2006**, 103(27):10461–10466.
[45] Kittakoop P, Mahidol C, Ruchirawat S. *Current Topics in Medicinal Chemistry*, **2014**, 14(2):239–252.
[46] Cushnie TT, Cushnie B, Lamb AJ. *International Journal of Antimicrobial Agents*, **2014**, 44(5):377–386.
[47] Shi Q, Hui S, Zhang A, Hong-Ying XU, Guang-Li Y, Ying H, Xi-Jun W. *Chinese Journal of Natural Medicines*, **2014**, 12(6):401–406.
[48] Sinatra RS, Jahr JS, Watkins-Pitchford JM. The Essence of Analgesia and Analgesics. Cambridge University Press, UK. **2011**, 515.