

Synergic anti-oxidant efficiency of ginger and green tea phytomolecular complex

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ABSTRACT

Phytochemicals are natural compounds widely distributed in the plant sources. A plant food is an emerging sector to heal the human systems through its phytochemical composition. The interactions of the molecules in the ginger and green tea extracts have the tendency to change the functional property in terms of synergism towards anti-oxidant activity. In this research study, an understanding about anti-oxidant efficiency of ginger and green tea phytomolecules was analysed individually and in combination to reveal the synergic effect of the beverage. The result of the study has revealed that, the combination of ginger–green tea complex has positive synergic effect and its consumption can improve health.

Keywords: Antioxidants, Phytochemicals, Ginger, Green tea, Synergism

INTRODUCTION

A food nourishes the living organisms with essential nutrients. It contains macro and micromolecules which aid in sustaining the homeostasis of the organism. The macromolecules present in our diet, such as carbohydrates, proteins, lipids and nucleic acids are involved in a majority of physiological functions [1]. The micromolecules have the scope in regulating the cell signaling and its metabolic functions [2]. Plants are predominant source of macromolecules and they also contribute a wide range of micromolecules, which are naturally enriched with medicinal values and found to be more interactive with regular physiological functions of the human body. These micromolecules are usually termed as phytochemicals or phytomolecules [3]. These biomolecules present in the plant foods have a variety of scope in interacting with each other and have the tendency to alter the overall activity of the individual molecules. This alteration could result in increase in the overall activity of the molecule(s), which is termed as synergic and the reversal of the same is antagonistic [4]. On a positive approach, different formulation of the plant (micromolecular) components could potentially increase the efficiency of the end product. Hence, an attempt has been made in this research to develop a functional drink with the composition of green tea and ginger phytomolecules. Green tea is widely consumed as a drink [5] which is derived from the leaves of the plant, *Camellia sinensis* and it differs from other drinks due to its phytochemical composition enriched with polyphenolic compounds [6] that are significantly involved in the anti-oxidant activity. The rhizome of *Zingiber officinale* (usually termed as ginger) originated from the species Zingiberaceae [7] is enriched with phytochemicals like gingerols, shogaols, gingerone, etc. and is found to produce some principle therapeutical values including anti-oxidant activity [8]. It is widely used as culinary spice [9]. The major objective of the study is to analyse the anti-oxidant efficiency of green tea and ginger extracts individually and in the combined conditions, and to reveal the synergic efficiency of the functional ginger–green tea beverage.

MATERIALS AND METHODS

Plant materials and chemicals

Green tea leaves and ginger were purchased from the local market. All the chemicals used in the study were obtained from Merck Chemicals, India.

Extraction of phytomolecular complex from green tea using different solvents

Green tea leaves were finely powdered. 1 gram of the leaf powder was weighed and taken in a glass beaker. It was mixed with deionised water in the concentration of 1:25. The mixture was kept in a thermostatic water bath at 80°C for 40 min. The contents of the beaker were then centrifuged at 3000 rpm for 20 min and the supernatant was collected. The supernatant of each extract was then treated in a rotary vacuum evaporator to remove the solvent. The condensed material was dissolved in 50 ml of deionised water and used for further experiments [10]. The experiment was repeated with ethanol as the solvent [11].

Extraction of phytomolecular complex from ginger using different solvents

Fresh ginger was peeled, washed thoroughly and finely chopped. 1 g of chopped ginger was weighed and ground well with 10 ml of distilled water using mortar and pestle into fine paste. The paste was then mixed with 15 ml of distilled water and stirred well using a cyclomixer for 10 min. The mixture was then kept in a thermostatic water bath at 80°C for 40 min. The contents were then centrifuged at 3000 rpm for 20 min and the supernatant was then subjected to rotary vacuum evaporator treatment for solvent removal. Further, the condensed material was mixed with 50 ml of deionised water and used for further experiments. The experiment was repeated with ethanol as the solvent [11].

Test sample preparation

Table 1. The test samples were prepared in the following composition

Test sample code	Sample composition
AG ₁	1 ml of aqueous ginger extract
AG ₂	1 ml of aqueous green tea extract
AGG ₃	0.5 ml of aqueous green tea extract mixed with 0.5 ml of aqueous ginger extract
EG ₁	1 ml of ethanolic ginger extract
EG ₂	1 ml of ethanolic green tea extract
EGG ₃	0.5 ml of ethanolic green tea extract mixed with 0.5 ml of ethanolic ginger extract

Evaluation of Ferrous Reducing Power

1 ml of the test sample was taken in a clean tube and added with 2.5 ml of phosphate buffer (0.2 M) and 2.5 ml of potassium ferricyanide (1%) and mixed well using a vortex mixer. The mixture was then kept at 50°C in a hot air oven for 30 min. After incubation, the mixture was allowed to cool in room temperature and 2.5 ml of (10%) trichloroacetic acid was added and subjected to centrifugation at 3000 rpm for 10 min. 2.5 ml of the supernatant was carefully taken and mixed with 2.5 ml of distilled water and mixed well. 0.5 ml of freshly prepared ferric chloride solution was added and mixed well and the absorbance was measured at 700 nm. Control was prepared in similar manner where the test sample is replaced with distilled water. The procedure was repeated for all the test samples and the experiments were conducted in triplicate, and the results were tabulated [12].

Evaluation of hydroxyl radical scavenging efficiency

Test sample (1 ml) was mixed with 1 ml of ferrous sulphate (1.5 mM), 0.7 ml of 6 mM hydrogen peroxide and 0.3 ml of 20 mM sodium salicylate. The text mixture was labelled as (X₁). The text mixture made without sodium salicylate was labelled as (X₂). The text mixtures were kept at 37°C for 60 min. After incubation, the absorbance was measured at 562 nm in a visible spectrophotometer. Control experiment was made by replacing the test sample with distilled water. Hydroxyl scavenging activity was calculated using the formula [13]:

$$\text{Scavenging activity} = 1 - \frac{(X_1 - X_2)}{X_1} \times 100$$

Evaluation of hydrogen peroxide scavenging efficiency

300 µl of test sample was mixed with 1200 µl of 40 mM hydrogen peroxide solution. The mixture was incubated at room temperature for 10 min and the absorbance was measured using UV-Visible spectrophotometer at 230 nm against phosphate buffer as blank. The percentage of scavenging of hydrogen peroxide of extracts and standard compounds was calculated using the formula [14]:

$$\% \text{ hydrogen peroxide scavenging} = \frac{A_c - A_s}{A_c} \times 100$$

Where, “Ac” is the absorbance without samples and “As” denotes absorbance in the presence of the samples.

RESULTS AND DISCUSSION

Synergy is the condition when the interaction of two substances causes increase in the effect of one or both. When the interaction of two substances causes decrease in the effect of one or both the substances, such interaction is said to be antagonistic. In this research study, phytochemical complex from ginger rhizome and green tea leaves was extracted using different solvents (water and ethanol) and the samples were tested for individual anti-oxidant potential and for combined effect of the same. When two biologically active compounds are combined together, they can either produce synergistic or antagonistic effect in the overall result of their therapeutical efficiency. The major motive of the study strived towards measuring the combined effect of ginger and green tea extracts.

Reducing power of ginger–green tea complex

The anti-oxidant properties of the polyphenol present in the extracts were majorly due to its molecular integrity [15]. The reducing ability was assayed using FRAP method for both aqueous and ethanolic extract. The result indicates that, ginger extract has chelating properties that would result in the reduction of Fe (III) to Fe (II); the complex formation is disrupted with the result of colour reduction. The hydroxyl group is responsible for the radical chelating property [16]. The chelating activity was then measured; it was found that, the aqueous and ethanol green tea extract had shown (70.9%) and later (75.5%). Ginger extract had shown least in aqueous (80.64%) and higher in ethanolic (88.78%). In comparison to earlier extract, the combination of ginger–green tea complex possessed maximum synergic effect in both solvent aqueous (90.5%) and ethanolic extract (95.4%) as mentioned in table 2 and 3.

Table 2. Reducing efficiency of aqueous extracts

Optical Density (OD)	AG ₁	AG ₂	AGG ₃
700 nm	70.9%	80.64%	90.5%

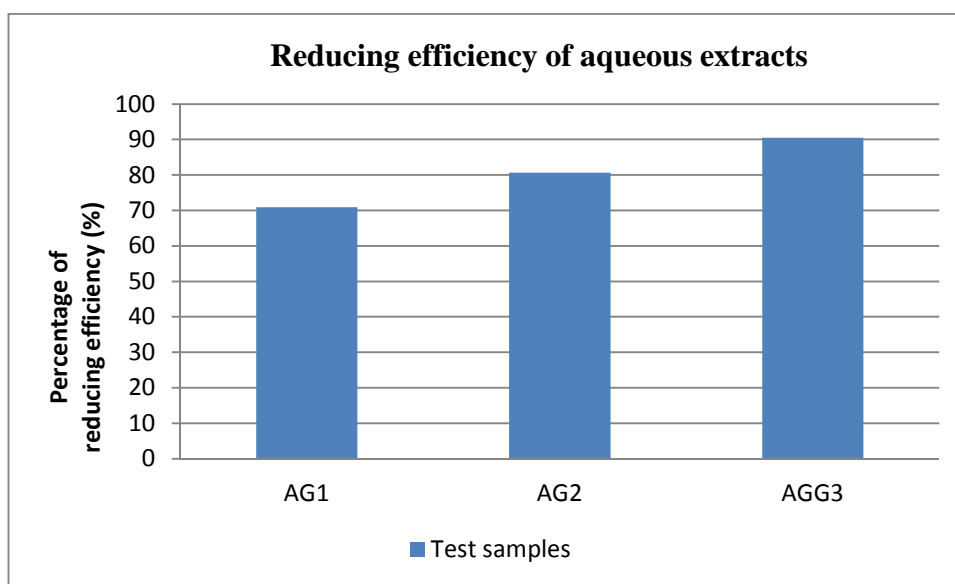
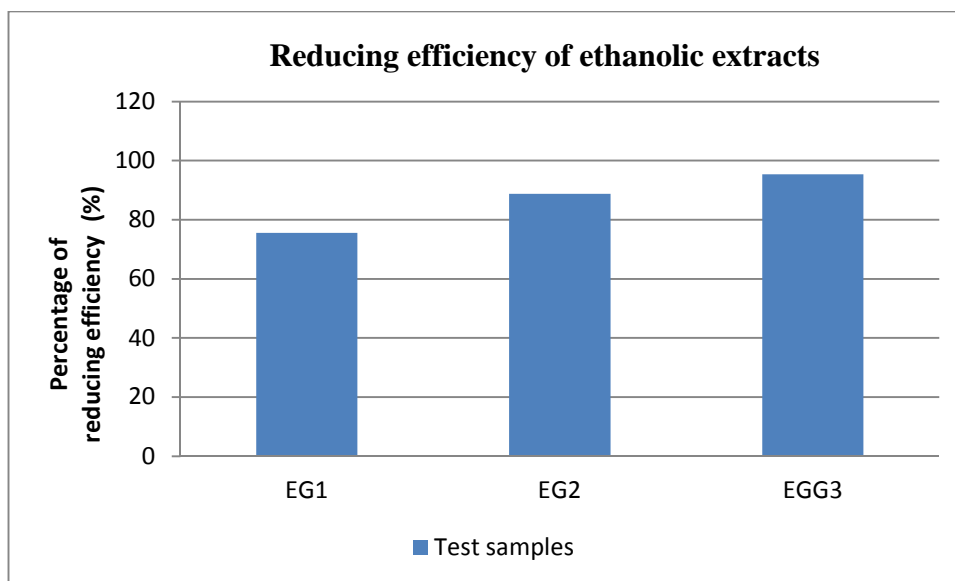


Table 3. Reducing efficiency of ethanolic extracts

Optical Density (OD)	EG ₁	EG ₂	EGG ₃
700 nm	75.5%	88.78%	95.4%



Hydroxyl radical scavenging activity of ginger–green tea complex

Hydroxyl radical is highly reactive species and consequently short-lived; it forms the neutral form of hydroxide ion that oxidises the organic compounds and chelates the ions. The molecules with scavenging abilities of the extract react with the unstable hydroxyl radicals in the presence of sodium salicylate to form a stable complex which is measured spectrophotometrically at 562 nm [13]. The scavenging ability of the aqueous extract was higher than the ethanolic extract and is due to the presence of the functional components [17]. The polyphenolics with the functional group of hydroxyl influence more scavenging activity [18]. The AGG₃ (90.3%) and EGG₃ (99.8%) efficiency in scavenging the free radicals was relatively higher when compared with AG₁ (98.9%), AG₂ (77.2%), EG₁ (92.6%) and EG₂ (82.6%) as depicted in table 4 and 5.

Table 4. Hydroxyl radical scavenging efficiency of aqueous extracts

Optical Density (OD)	AG ₁	AG ₂	AGG ₃
562 nm	98.9%	77.2%	90.3%

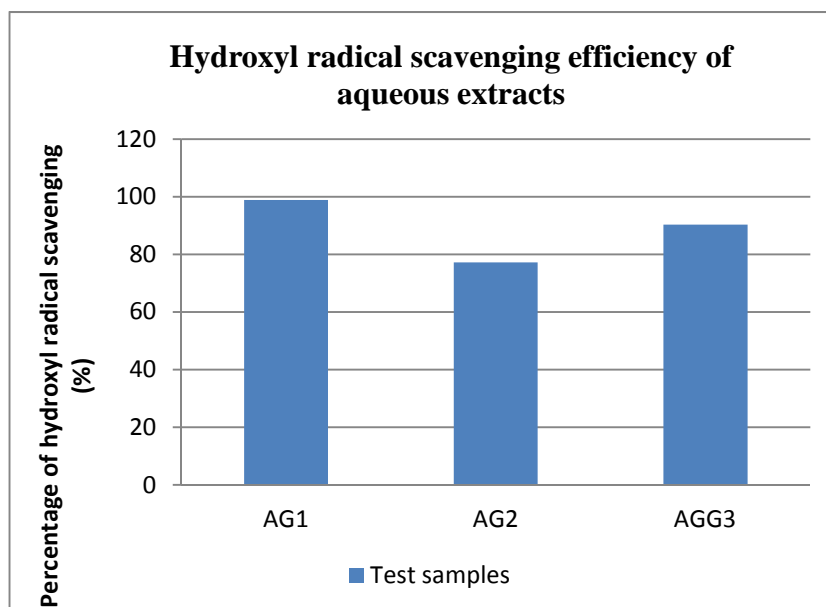
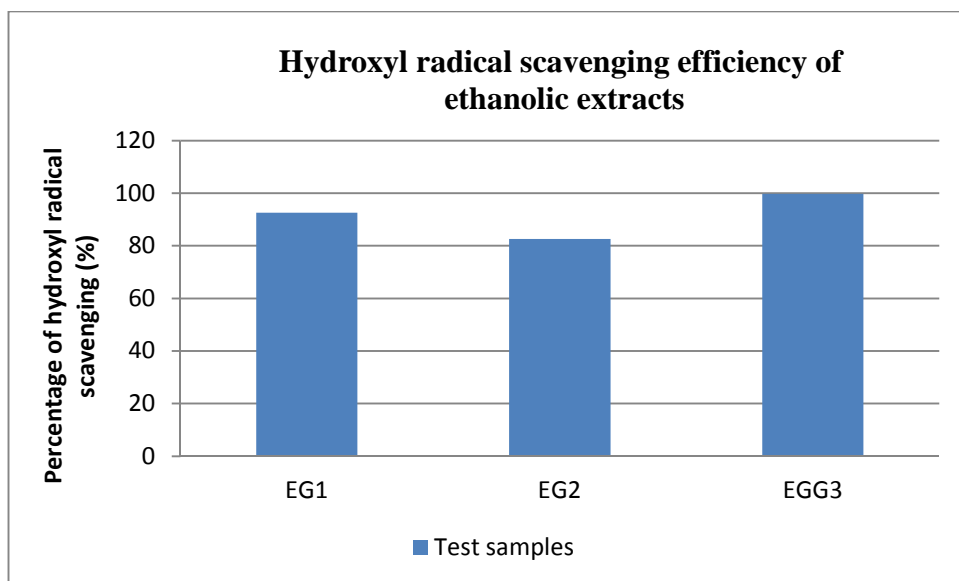


Table 5. Hydroxyl radical scavenging efficiency of ethanolic extracts

Optical Density (OD)	EG ₁	EG ₂	EGG ₃
562 nm	92.6%	82.6%	99.8%



Hydrogen peroxide scavenging activity ginger–green tea complex

Hydrogen peroxide is a potential oxidant of organic compounds. The scavenging activities of the extracts on hydrogen peroxide were analysed. The result was found to be higher efficiency in the combination of ginger–green tea extract in comparison to scavenging efficiency of individual extract [19]. The ethanol extract was found to be higher (98.5%) than the aqueous extract (96.62 %). The combination of extract was tremendously effective in comparison to the earlier (table 6 and 7). The individual aqueous extract of ginger had shown higher activity (93.98%) in comparison to green tea (86.55%); whereas, the ethanolic extract also resulted maximum activity in ginger extract (94.12%) than green tea (89.20%) extract [20].

Table 6. Hydrogen peroxide scavenging efficiency of aqueous extracts

Optical Density (OD)	AG ₁	AG ₂	AGG ₃
230 nm	93.98%	86.55%	96.62%

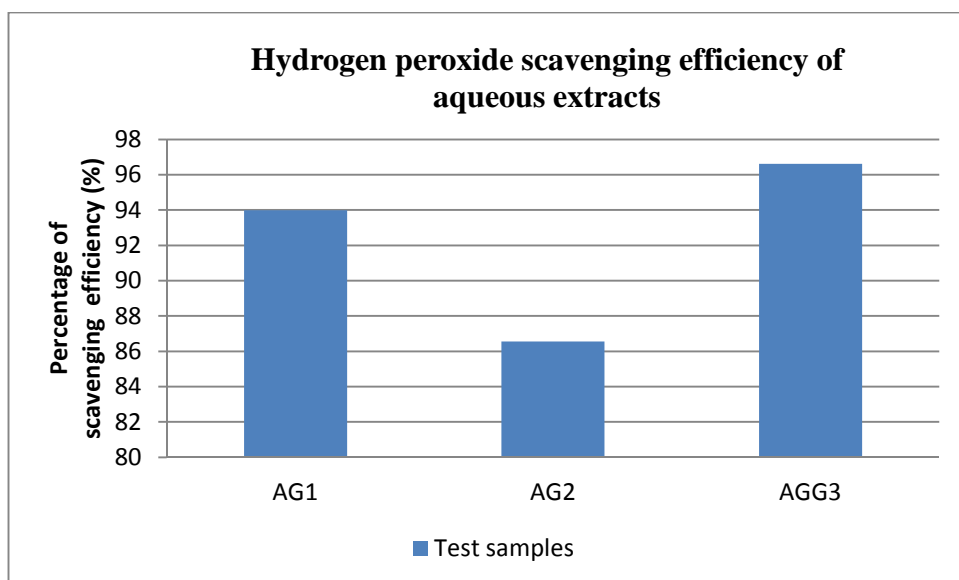
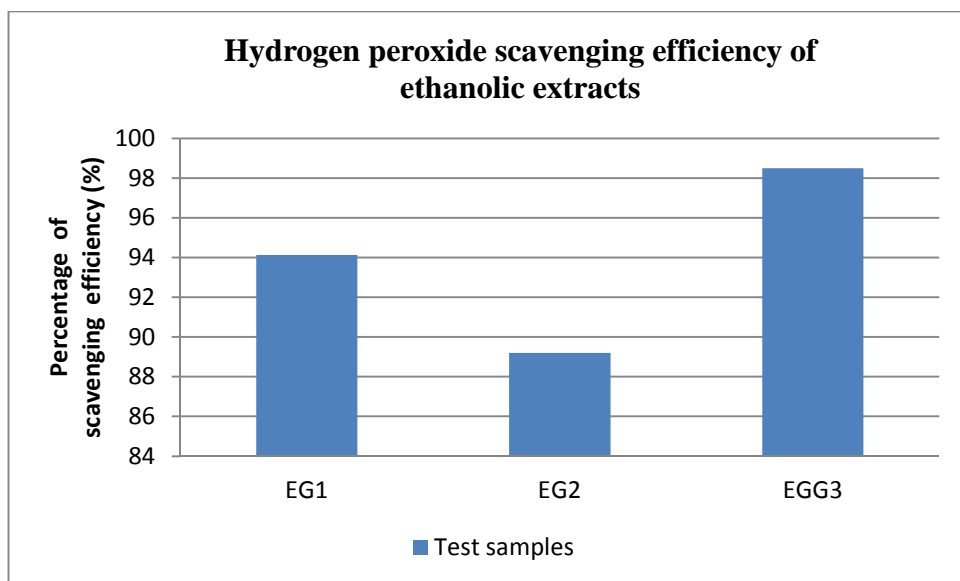


Table 7. Hydrogen peroxide scavenging efficiency of ethanolic extracts

Optical Density (OD)	EG ₁	EG ₂	EGG ₃
230 nm	94.12%	89.20%	98.5%



Synergism between phytochemicals is greatly possible because of the presence of diversified molecules in the plants. Combinations of different molecules or total extract provide better therapeutic efficiency rather than isolated pure molecules [21]. Improved effects were seen in cytotoxicity and antitumor activity of total extract of *Punica granatum* (pomegranate) rather than its isolated polyphenols [22]. Extracts of ginseng and ginkgo show improved cognitive function rather than the individual compounds [23]. Phytotherapeutics bring out different approach in healthcare. Combination of antibiotics with plant molecules increases the antimicrobial activity; methicillin resistant *Staphylococcus aureus* had shown sensitivity against the combination of *Cordia gillettii* and tetracycline [24]. Similarly, 15 different plants combined with tetracycline display improved activity against *Escherichia coli* [25][26]. Antifungal activity is improved by combining amphotericin B with epigallocatechin gallate, a polyphenol isolated from green tea [25]. Combination of oregano and mulberry extracts was effective against *Vibrio parahaemolyticus* and *Helicobacter pylori* [27][28]. Multi combination of phytotherapeutics may expel drastic betterment in the pharmaceutical world in near future. In our work, the synergistic activity of the extracts is due to the cumulative effects produced by interactions between the compounds present in the herbs between phytochemicals of *Camelia sinensis* (Green tea) and *Zingiber officinale* (ginger); the interaction between them resulted in protecting the active substances from decomposition; thus, the overall effects increased the anti-oxidant efficiency [4].

CONCLUSION

The current research was carried out to understand the functionality of green tea and ginger phytochemicals, independently or in combination, in relevant to anti-oxidant properties. The short research identified the existence of synergism between the molecules of the phyto mixture which has increased the anti-oxidant efficiency of the mixture. The current study showcases the possibility of improving the anti-oxidant efficiency of a morning green tea drink enriched with ginger. Further research insights must be produced to measure further activities of the combinations and reasoning of the same. The research should be driven on evaluation of all the possible side effects that would be occurring when the effects are increased.

REFERENCES

- [1] Shubhrata MR, *Biomolecules*, Discovery Publishing House, New Delhi, **2003**.
- [2] Gumbiner BM, *Cell*, **1996**, 84, 345–357.
- [3] Belobrajdic DP, Bird AR, *Nutr J*, 2013, 12, 2–12.
- [4] Biavatti M, *Braz J Pharma Sci*, **2009**, 45, 372–378.
- [5] Subhashini R, MahadevaRao US, Sumathi P, Gunalan G, *Indian J Sci Technol* **2010**, 3, L188–192.
- [6] Hilal Y, Engelhardt U, *J Verbrauch Lebensm*. **2007**, 2, 414–421.
- [7] Indrayan AK, Agrawal P, Rathi AK, Shatru A, Agrawal NK, Tyagi DK, *Nat Prod Rad*, **2009**, 8, 507–513.
- [8] Malhotra S, Singh AP, *Nat Prod Rad*. **2003**, 2, 296–301.
- [9] Ghasemzadeh A, Hawa ZE Jaafar, *J Med plants Res*, **2011**, 5(14), 3247–3255.
- [10] Uzunalic PA, Skerget M, Knez Z, Weinreich B, Otto F, Grucher S, *Food Chem*, **2006**, 96, 597–605.
- [11] Gupta RK, Chawla P, Tripathi M, Shukla AK, Pandey A, *Int J Pharm Pharm Sci*, **2014**, 6, 477–479.
- [12] Benzie IF, Strain JJ, *Anal Biochem*, **1996**, 239, 70–76.

- [13] Subramanian S, Vadanarayanan S, *Int Res J Pharm*, **2012**, 3, 3984–398.
- [14] Ruch RJ, Cheng SJ, Klaunig JE, *Carcinogenesis*, **1989**, 10, 1003–1008.
- [15] Naczek M, Amarowicz R, Zadernowski R, Pegg RB, Shahidi F, *Pol J Food Nutri Sci*, 2003, 12, 166–169.
- [16] Selvakumar K, Madhan R, Srinivasan G, Baskar V, *Asian J Pharm Tech*, **2011**, 1(4), 99–103.
- [17] Aparadh VT, Naik V, Karadge BA, *Ann Bot*, **2012**, 2, 49–56.
- [18] Motlhanka DMT, Habtemariam S, Houghton P, *Afr J Biomed Res*, **2008**, 11, 55–63.
- [19] Zepp RG, Bruce C, Faust, Hoigne J, *Env Sci Technol*, **1992**, 26, 313–319.
- [20] Asimi OA, Sahu NP, Pal AK, *Int J Sci Res Publ*, **2013**, 3, 1–8.
- [21] Nelson AC, Kursar TA, *Chemoecology*, **1990**, 9, 81–92.
- [22] Seeram NP, Adams LS, Henning SM, Niu Y, Zhang Y, Nair MG, Heber D, Navindra P, *J Nutr Biochem*, **2005**, 16, 360–367.
- [23] Williamson EM, *Phytomedicine*, **2001**, 8, 401–408.
- [24] Okusa PN, Penge O, Devleeschouwer M, Dueza P, *J Ethnopharmacol*, **2007**, 112, 476–481.
- [25] Ahmad I, Aqil F, *Microbiol Res*, **2007**, 162, 264–275.
- [26] Han Y. *Biol Pharm Bull*, **2007**, 30, 1693–1696.
- [27] Lin YT, Labbe RG, Shetty K, *Innova Food Sci, Emerg, Technol*, **2005**, 6, 453–458.
- [28] Vattem DA, Lin YT, Ghaedi R, Shetty K, *Process Biochem*, **2005**, 40, 1583–1592.