# Supplementary File S1

## Bioinformatic insights on biochemical efficacy of a fungal metabolite: Asperyellone

**Iswarya Santhakumaran[1], Radhakrishnan Narayanaswamy[2], Gnanamani Arumugam[1*]**

[1] Microbiology Division, CSIR-CLRI, Adyar, Chennai-600 020, Tamil Nadu, India.

[2] St. Peter's Institute of Higher Education and Research, Avadi, Chennai-600 054, Tamil Nadu, India.

***Author for Correspondence**

Dr. A. Gnanamani,

Microbiology Division,

CSIR- CLRI, Adyar,

Chennai - 20,

Tamil Nadu, India.

Email: gnanamani3@gmail.com

## Materials and methods:

### Optimization of growth medium

In order to understand the role of carbon, nitrogen and physical parameters on the production of Asperyellone, experiments were carried out individually with four different concentrations (0.5, 1.0, 1.5, 2.0%) of carbon (glucose, fructose, sucrose, dextrose and maltose) sources and nitrogen (yeast extract, beef extract, peptone, sodium nitrate) sources, at varied pH conditions (3.0, 4,0, 5.0, 6.0 and 7.0), at varied temperature conditions (25, 30, 35, 40, and 45º C) and at different agitation speed (50, 100, 150, 200 and 250 RPM).

### Assessment of Reducing property

### Reducing power of AY

Reducing power of AY sample determined according to the method described [1] with some modifications. Briefly, various concentration of the AY sample mixed with 0.2M sodium phosphate buffer (pH 6.6) and 2.5ml of 1% potassium ferricyanide. The mixture was then incubated at 50ºC for 20 minutes. Followed by incubation added 2.5ml of 10% trichloroacetic acid and then centrifuged at 6500 RPM for 10 minutes. A portion of the supernatant diluted with deionized water, mixed with 1ml of 0.1% ferric chloride and measured the absorbance was at 700nm. Ascorbic acid was used as standard.

### Ferrous ion chelation ability of AY

The ability of AY sample to chelate ferrous ion estimated according to the method described [2] with some modifications. Briefly, 1ml of various concentration of the AY sample mixed with 0.25ml of 2mM $FeCl_2$. Then, 0.1ml of 5mM Ferrozine was added to initiate the reaction and the mixture was shaken vigorously at room temperature for 10min and then measured the absorbance at 562nm. Ascorbic acid was used as standard. The percentage inhibition was calculated using the following equation.

$$\% \text{ Inhibition} = \frac{(A0 - A1)}{A0} * 100$$

Where, $A_0$ = Absorbance of the control sample, $A_1$ = Absorbance of the test sample

### Assessment of Antioxidant property

### Hydrogen peroxide scavenging ability of AY

The ability of AY sample to scavenge the hydrogen peroxide radical estimated according to the method described [3] with some modifications. Briefly, various concentrations of the AY sample in methanol (0.5ml) was added to 1ml of Hydrogen peroxide (20mM) in PBS, incubated for 10 min. and then measured the absorbance at 230nm. Ascorbic acid was used as a standard. The percentage inhibition was calculated using the following equation.

$$\% \text{ Inhibition} = \frac{(A0 - A1)}{A0} * 100$$

Where, $A_0$ = Absorbance of the control sample, $A_1$ = Absorbance of the test sample

**Nitric oxide radical scavenging property of AY**

The quenching of nitric oxide radical by AY sample estimated by the method described [4] with some modifications. Briefly, 10 mM Sodium nitroprusside prepared using phosphate buffered saline (pH 7.4). Griess reagent was prepared by mixing 0.1 % α- Naphthyl ethylenediamine in sterile water and 1% Sulphanilic acid in 5% $H_3PO_4$. Various concentration of AY sample mixed with 1ml of sodium nitroprusside and the reaction mixture was incubated at 25º C for 60min. To the mixture, 1.5ml of Griess reagent added and measured the absorbance at 546 nm after 30min incubation. Ascorbic acid was used as a standard. The percentage inhibition of nitrite ions generated was calculated using the following equation.

$$\% \text{ Inhibition} = \frac{(A0 - A1)}{A0} * 100$$

Where, $A_0$ = Absorbance of the control sample, $A_1$ = Absorbance of the test sample

**Superoxide anion radical scavenging property of AY**

The quenching ability of AY sample against superoxide anion radicals described [4] with some modifications. In brief, various concentrations of AY sample added with 0.1M Tris HCl (pH 8), 1ml NADH (1mM), 1ml Nitro Blue Tetrazolium (0.5mM) and 1ml of Phenazine Metho Sulphate (0.1mM). The reaction mixture was incubated at 25º C for 5 min. The absorbance was measured at 560nm. Ascorbic acid used as a standard. The % inhibition calculated using the following equation,

$$\% \text{ Inhibition} = \frac{(A0 - A1)}{A0} * 100$$

Where, $A_0$ = Absorbance of the control sample, $A_1$ = Absorbance of the test sample.

**Statistical Analysis**

Statistical analysis was performed using one-way ANOVA and the pair comparison by Turkey test. The level of significance was represented for three different significant levels: high significance (***P<0.001), significant (**P<0.01) and less significant (*P<0.05).

## Results and Discussion

Of all the carbon sources studied, sucrose at 1.5%, followed by glucose at 2% concentration observed to be favorable for the maximum pigment production as shown in Figure S1A(a), and with respect to nitrogen source, sodium nitrate enhances the pigment production at 1% concentration followed by peptone at 1.5% as shown in Figure S1A(b). With respect to environmental parameters, pigment production found maximum at agitation speed of 150 RPM, agitation speed higher than 150 RPM significantly (P<0.05) decreases the pigment production (Figure S1B(a). The optimum pH and temperature favor the pigment production observed as 5.0 and 25º C respectively (Figure S1B(b and c). The optimum incubation period for Asperyellone pigment production determined as 96 hr. as shown in Figure S1B(d), and further increase in incubation period, reduces the pigment yield. A study on *A.niger* strain reports, that dextrose, sucrose and $NaNO_3$ supplementation favors maximum pigment production of the pigment [5]. In addition, available reports suggested that several ingredients in minimum quantity enhance the pigment production, which includes phosphate, diphenyl, thiamin, riboflavin, p- amino benzoic acid and n-butyl p- aminobenzoate [6-9], but in the present study, no additional ingredients were employed. Since, pigments and other secondary metabolites are pH sensitive and work well at optimum pH [10] and temperature, in the present study, maximum production yield of the pigment observed at optimum pH (5.0) and temperature (25° C) which corroborates well with the available reports. It was reported that inoculum age plays a major role in the development of fungus[11] and in the present study slight pigmentation was observed on day 7[12].

**Reducing property of Asperyellone**

Chemical species with one or more unpaired electrons is called as free radicals, or ROS/RNS, Reactive oxygen species /Reactive nitrogen species (RNS). These radicals are highly reactive and are continuously produced during the metabolism in human body, and are essential for the process of detoxification, immune function, chemical signaling, etc. These radicals stabilized by antioxidant

molecules [13] and thus antioxidants prevent the damaging effect caused by the free radicals. However, the excess ROS/RNS generation leads to an imbalance between the natural antioxidants and free radicals, which lead to oxidative stress[14]. In case of reducing power assay, the substance with reducing property reacts with potassium ferricyanide (3+) and gets converted to potassium ferrocyanide (2+). This reacts with ferric chloride to form ferric ferrous complex, which can be absorbed in 700 nm. The reducing power of AY as a function of concentration was shown in Figure S2A(a) and suggests the reducing power of AY increases with an increase in concentration of AY sample. AY sample at a maximum concentration of 30 µg showed absorbance of 2.5 whereas, Ascorbic acid at 50 µg showed absorbance of 1.65. The observed results in the present study on the reducing power property of asperyellone matches with the report[15] wherein, the authors studied on anthocyanins extracted from the fruits of *Terminalia cattapa* L. The reducing power of extract of *Phellinus meriillii*[16], extracts of water hyacinth[17], purified fractions of *Salvia mirzayanii*[1], and methanolic extract of *Desmodesmus sp.*[18] were also in reports.

Metal ion chelation assay determines the nature of compound to reduce transition metals that catalyze the lipid peroxidation by Fenton reaction. Ferrozine used in the assay forms complex with the Fe2+ and produces red color. In the presence of samples with chelating property, the complex formation will get disrupted and it will be observed by a decrease in color. This reduction in color is directly proportional to the chelating effect of the samples tested. The chelation power of AY sample estimated as 68.7% at maximum concentration of 50µg, whereas ascorbic acid at same concentration showed only 52.5% as shown in Figure S2A(b). The IC50 value of AY estimated as 35± 2µg. The study on metal chelation property of the carotenoid pigment extracted from *Sporobolomyces* sp. reported metal chelation rate of about 59.32% at 100 µg/ml concentration . The red pigment isolated from *Penicillium purpurogenum* showed 51.37% chelation at minimal concentration of 20 mg/ml[18]. Similarly, anthocyanins from the pericarp of *Terminalia cattapa* L fruits showed good metal chelation activity and the IC50 estimated as 86µg/ml, whereas ascorbic acid showed reduction rate of 65.2% at 100µg/ml concentration[15]. Pigments isolated from the *Desmodesmus* sp. showed highest reduction rate of 20% at minimal concentration of about 1mg/ml concentration[18]. The chelation rate of the mycelia extracts of *Volvariella volvacea* showed 50% iron chelation at a minimal concentration of about 0.88mg/ml[2]. All the summarized results support the observations on metal chelation efficacy of Asperyellone of the present study.

**Antioxidant property of Asperyellone**

With respect to hydroxyl radicals, the decomposition of hydrogen peroxide to oxygen and water, which leads to the generation of hydroxyl radicals. These hydroxyl radicals will create adverse effects like peroxidation of membrane lipids and cause DNA damage. The ability of the compound in scavenging the hydroxyl radicals was estimated by measuring the absorbance at 230nm. With respect to scavenging of hydroxyl radicals, AY sample at a concentration of 50µg/ml scavenges 74% of hydroxyl radicals, whereas ascorbic acid showed only 50% scavenging effect at that concentration (Figure S2B(a)) and the IC50 value of AY estimated as 30±1 µg. The present findings on hydroxyl radical scavenging of the pigment Asperyellone correlates with the reports on anthocyanins[15] of *Terminalia cattappa* L. and aqueous extract of *Volvariella volvaceai* [2].

With respect to NO radical scavenging potential of AY, about 60% of radicals scavenged by AY at a maximum concentration of 50 µg/ml, whereas ascorbic acid showed 71% inhibition at the same concentration (Figure S2B(b)) and the IC50 value of AY determined as 42±1.5µg. Figure S2B(c)) illustrates superoxide anion scavenging property of AY sample and showed 58.5% scavenging at the maximum concentration of 50 µg/ml, whereas standard ascorbic acid showed 68% inhibition at the same concentration and the IC50 value of AY calculated as 45±1.5 µg. The pigment asperyellone showed appreciable scavenging profile of superoxide anion radical and NO radical which correlates well with the results on methanolic extract of *Indigofera cassioides* [19], methanolic extract of *Caesalpinia digyna* root[3] and the extract from the stem bark of *Spondias pinnata*. The superoxide anion scavenging potential of stem bark extract of *Spondias pinnata*, reported with an IC50 value of 14 µg/ml, which is significantly lower than the standard quercetin which showed 43 µg/ml[20]. Similarly, the scavenging property of methanolic extract of *Indigofera cassioides* against superoxide anion radical estimated with an IC50 value of 232 µg/ml[19]. The methanolic extract of *Caesalpinia digyna* root showed good superoxide anion radical scavenging capacity with an IC50 value of 820 µg/ml[3].

## References


1. M.R. Moein, S. Moein and S. Ahmadizadeh, *Molecules*, 2008, **13**, 2804-13.
2. V. Kalava and S. Menon, *Int. J. Curr. Pharm. Res*, 2012, **4**, 1-6.
3. R. Srinivasan, M.J.N. Chandrasekar, M.J. Nanjan and B. Suresh, *J. Ethnopharmacol*, 2007, **113**, 284-291.
4. V. Sasikumar and P. Kalaisezhiyen, *Cogn, Biochem. Anal. Biochem*, 2014, **4**, 42-46.
5. J.E. Zajic, H.H. Kuehn, *Mycopathol,* 1962, **17**, 149-158.
6. W.G. Solheim, S.S. Sears and R.C. Rollins, *Phytopathol*, 1933, **23**, 929-930.
7. M.R. Hertz and M.A. Levine, *Food Res,* 1942, **7**, 430-441.
8. T. Mann, Studies on the metabolism of mold fungi. *Biochem. J*, 1944, **38**, 339-345.
9. G.W.K. Cavill and J.M. Vincent, *Nature*, 1945, **155**, 301.
10. A. Sabu, G.S. Kiran and A. Pandey, *Food. Tech. Biotech*, 2005, **43**, 133-138.
11. J.T. Bae, J. Sinha, J.P. Park, C.H. Song and J.W. Yun, *J. Microbiol. Biotech*. 2000, **10**, 482-487.
12. T.L. Szymanska and E. Galas, *Enz Microbia. Tech*, 1993, **15**, 317-320.
13. M.A. Ebrahimzadeh, F. Pourmorad and S. Hafezi, Antioxidant activities of Iranian corn silk, *Turk. J. biol*, 2008, **32**, 43-49.
14. H.E. Miller, F. Rigelhof, L. Marquart, A. Prakash and M. Kanter, *J. American. Coll. Nut*, 2000, **19**, 312S-9S.
15. M.R. Prabha, *Int. J. Pharma. Biosci*, 2015, **6**, 72-76.
16. H.Y. Chang, Y.L. Ho, M.J. Sheu, Y.H. Lin, M.C. Tseng, S.H. Wu, G.J. Huang and Y.S. Chang, *Bot. Stud,* 2007, **48**, 407-417.
17. P. Jayanth and P. Lalitha, *Int. J. Pharm. Pharmaceu. Sci*, 2011, **3**, 126-128.
18. H. Safafar, J. Van Wagenen, P. Moller and C. Jacobsen, *Maraine drugs*, 2015, **13**, 7339-7356.
19. R. Senthil Kumar, B. Rajkapoor and P. Perumal, *Asian. Pac. J. Trop. Med*, 2012, **2**, 256-261.
20. B. Hazra, S. Biswas and N. Mandal, *BMC Comp. Alt. Med,* 2008, **8**, 63.

195 **Table S1:** Geometrical parameters [bond length (Å), bond angle ( ) and dihedral angle ( )] of

196 Asperyellone

| Bond length | B3LYP/613-G (d,p) | Bond angle | B3LYP/613-G (d,p) | Dihedral angle | B3LYP/613-G (d,p) |
|---|---|---|---|---|---|
| C1-C2 | 1.406 | C2-C1-C6 | 121.3 | C6-C1-C2-C3 | 0.0 |
| C1-C6 | 1.390 | C2-C1-H7 | 119.0 | C6-C1-C2-C12 | -180.0 |
| C1-H7 | 1.085 | C6-C1-H7 | 119.7 | H7-C1-C2-C3 | 180.0 |
| C2-C3 | 1.408 | C1-C2-C3 | 117.7 | H7-C1-C2-C12 | 0.0 |
| C2-C12 | 1.460 | C1-C2-C12 | 118.8 | C2-C1-C6-C5 | 0.0 |
| C3-C4 | 1.388 | C3-C2-C12 | 123.5 | C2-C1-C6-H11 | -180.0 |
| C3-H8 | 1.084 | C2-C3-C4 | 120.9 | H7-C1-C6-C5 | -180.0 |
| C4-C5 | 1.396 | C2-C3-H8 | 120.0 | H7-C1-C6-H11 | 0.0 |
| C4-H9 | 1.084 | C4-C3-H8 | 119.1 | C1-C2-C3-C4 | 0.0 |
| C5-C6 | 1.393 | C3-C4-C5 | 120.5 | C1-C2-C3-H8 | 180.0 |
| C5-H10 | 1.084 | C3-C4-H9 | 119.6 | C12-C2-C3-C4 | 180.0 |
| C6-H11 | 1.084 | C5-C4-H9 | 119.9 | C12-C2-C3-H8 | 0.0 |
| C12-H13 | 1.089 | C4-C5-C6 | 119.5 | C1-C2-C12-H13 | -0.2 |
| C12-C14 | 1.353 | C4-C5-H10 | 120.2 | C1-C2-C12-C14 | 179.7 |
| C14-H15 | 1.085 | C6-C5-H10 | 120.3 | C3-C2-C12-H13 | 179.8 |
| C14-C16 | 1.437 | C1-C6-C5 | 120.1 | C3-C2-C12-C14 | -0.2 |
| C16-H17 | 1.088 | C1-C6H11 | 119.8 | C2-C3-C4-C5 | 0.0 |
| C16-C18 | 1.364 | C5-C6-H11 | 120.1 | C2-C3-C4-H9 | 180.0 |
| C18-H19 | 1.084 | C2-C12-H13 | 114.9 | H8-C3-C4-C5 | 180.0 |
| C18-C20 | 1.433 | C2-C12-C14 | 127.8 | H8-C3-C4-H9 | 0.0 |
| C20-H21 | 1.086 | H13-C12-C14 | 117.3 | C3-C4-C5-C6 | 0.0 |
| C20-C22 | 1.368 | C12-C14-H15 | 119.3 | C3-C4-C5-H10 | 180.0 |
| C22-C23 | 1.508 | C12-C14-C16 | 122.8 | H9-C4-C5-C6 | -180.0 |
| C22-C27 | 1.445 | H15-C14-C16 | 117.9 | H9-C4-C5-H10 | 0.0 |
| C23-H24 | 1.095 | C14-C16-H17 | 115.4 | C4-C5-C6-C1 | 0.0 |
| C23-H25 | 1.088 | C14-C16-C18 | 127.7 | C4-C5-C6-H11 | 180.0 |
| C23-H26 | 1.095 | H17-C16-C18 | 116.9 | H10-C5-C6-C1 | -180.0 |
| C27-H28 | 1.088 | C16-C18-H19 | 116.3 | H10-C5-C6-H11 | 0.0 |
| C27-C29 | 1.359 | C16-C18-C20 | 126.2 | C2-C12-C14-H15 | 0.0 |
| C29-H30 | 1.086 | H19-C18-C20 | 117.5 | C2-C12-C14-C16 | -180.0 |
| C29-C31 | 1.435 | C18-C20-H21 | 116.5 | H13-C12-C14-H15 | 180.0 |
| C31-H32 | 1.088 | C18-C20-C22 | 127.6 | H13-C12-C14-C16 | 0.0 |
| C31-C33 | 1.351 | H21-C20-C22 | 115.9 | C12-C14-C16-H17 | -0.1 |
| C33-H34 | 1.087 | C20-C22-C23 | 123.7 | C12-C14-C16-C18 | 179.9 |
| C33-C35 | 1.480 | C20-C22-C27 | 117.9 | H15-C14-C16-H17 | 179.9 |
| C35-C36 | 1.523 | C23-C22-C27 | 118.4 | H15-C14-C16-C18 | 0.0 |
| C35-O39 | 1.221 | C22-C23-H24 | 110.7 | C14-C16-C18-H19 | -180.0 |
| C36-H37 | 1.092 | C22-C23-H25 | 112.7 | C14-C16-C18-C20 | 0.0 |
| C36-H38 | 1.096 | C22-C23-H26 | 110.7 | H17-C16-C18-H19 | 0.0 |
| C36-C40 | 1.539 | H24-C23-H25 | 107.7 | H17-C16-C18-C20 | 180.0 |
| C40-H41 | 1.093 | H24-C23-H26 | 107.1 | C16-C18-C20-H21 | 0.0 |
| C40-H42 | 1.094 | H25-C23-H26 | 107.7 | C16-C18-C20-C22 | 180.0 |
| C40-H43 | 1.093 | C22-C27-H28 | 115.8 | H19-C18-C20-H21 | 179.9 |
| | | C22-C27-C29 | 126.9 | H19-C18-C20-C22 | 0.0 |

| | | | |
|---|---|---|---|
| H28-C27-C29 | 117.3 | C18-C20-C22-C23 | 0.0 |
| C27-C29-H30 | 120.1 | C18-C20-C22-C27 | 180.0 |
| C27-C29-C31 | 122.7 | H21-C20-C22-C23 | 180.0 |
| H30-C29-C31 | 117.2 | H21-C20-C22-C27 | 0.0 |
| C29-C31-H32 | 117.8 | C20-C22-C23-H24 | -121.0 |
| C29-C31-C33 | 125.5 | C20-C22-C23-H25 | -0.3 |
| H32-C31-C33 | 116.6 | C20-C22-C23-H26 | 120.4 |
| C31-C33-H34 | 120.5 | C27-C22-C23-H24 | 58.9 |
| C31-C33-C35 | 121.4 | C27-C22-C23-H25 | 179.6 |
| H34-C33-C35 | 118.1 | C27-C22-C23-H26 | -59.6 |
| C33-C35-C36 | 116.8 | C20-C22-C27-H28 | 0.0 |
| C33-C35-O39 | 122.3 | C20-C22-C27-C29 | 180.0 |
| C36-C35-O39 | 120.9 | C23-C22-C27-H28 | -180.0 |
| C35-C36-H37 | 107.3 | C23-C22-C27-C29 | 0.0 |
| C35-C36-H38 | 109.7 | C22-C27-C29-H30 | 0.0 |
| C35-C36-C40 | 111.5 | C22-C27-C29-C31 | -179.9 |
| H37-C36-H38 | 108.4 | H28-C27-C29-H30 | 180.0 |
| H37-C36-C40 | 110.1 | H28-C27-C29-C31 | 0.0 |
| H38-C36-C40 | 109.7 | C27-C29-C31-H32 | -0.1 |
| C36-C40-H41 | 110.7 | C27-C29-C31-C33 | 179.7 |
| C36-C40-H42 | 111.7 | H30-C29-C31-H32 | 180.0 |
| C36-C40-H43 | 110.5 | H30-C29-C31-C33 | -0.3 |
| H41-C40-H42 | 107.8 | C29-C31-C33-H34 | 0.1 |
| H41-C40-H43 | 108.1 | C29-C31-C33-C35 | -179.4 |
| H42-C40-H43 | 107.9 | H32-C31-C33-H34 | 179.8 |
| | | H32-C31-C33-C35 | 0.3 |
| | | C31-C33-C35-C36 | -178.9 |
| | | C31-C33-C35-O39 | -0.2 |
| | | H34-C33-C35-C36 | 1.6 |
| | | H34-C33-C35-O39 | -179.7 |
| | | C33-C35-C36-H37 | -159.6 |
| | | C33-C35-C36-H38 | -42.0 |
| | | C33-C35-C36-C40 | 79.7 |
| | | O39-C35-C36-H37 | 21.7 |
| | | O39-C35-C36-H38 | 139.3 |
| | | O39-C35-C36-C40 | -99.0 |
| | | C35-C36-C40-H41 | 178.9 |
| | | C35-C36-C40-H42 | -61.0 |
| | | C35-C36-C40-H43 | 59.2 |
| | | H37-C36-C40-H41 | 59.8 |
| | | H37-C36-C40-H42 | 180.0 |
| | | H37-C36-C40-H43 | -59.9 |
| | | H38-C36-C40-H41 | -59.4 |
| | | H38-C36-C40-H42 | 60.8 |
| | | H38-C36-C40-H43 | -179.1 |

197

198

**Table S2:** Experimental and Calculated Absorption Wavelength, Energies and oscillator strengths of Asperyellone using the TD-DFT method of B3LYP/6-311G(d,p) method

| | Excitation | CI Expansion Coefficient | Wavelength λ (nm) | Oscillator Strength (f) | Expt. | Assign. | In Solvent [a] Major contribution (≥ 10%) |
|---|---|---|---|---|---|---|---|
| **Cal. DMSO phase** | **Excited State 1** | | | | | | |
| | 85 -> 86 | 0.70464 | 563.96 | 0.0006 | | π→π* | HOMO->LUMO(99%) |
| | **Excited State 2** | | | | | | |
| | 85 -> 87 | 0.66041 | 431.73 | 0.0033 | | π→π* | |
| | 85 -> 88 | -0.24168 | | | | π→π* | HOMO->L+1 (87%) HOMO->L+2 (12%) |
| | **Excited State 3** | | | | | | |
| **Cal. Gas phase** | 85 -> 87 | 0.24280 | 419.19 | 0.0033 | 425 | π→π* | HOMO->L+1 (12%) |
| | 85 -> 88 | 0.66242 | | | | π→π* | HOMO->L+2 (88%) |
| | **Excited State 1** | | | | | | |
| | 85 -> 86 | 0.70464 | 564.26 | 0.0006 | | π→π* | HOMO->LUMO(99%) |
| | **Excited State 2** | | | | | | |
| | 85 -> 87 | 0.66008 | 431.95 | 0.0010 | | π→π* | HOMO->L+1 (87%) |
| | 85 -> 88 | -0.24254 | | | | π→π* | HOMO->L+2 (12%) |
| | **Excited State 3** | | | | | | |
| | 85 -> 87 | 0.24371 | 419.44 | 0.0031 | | π→π* | HOMO->L+1 (12%) |
| | 85 -> 88 | 0.66209 | | | | π→π* | HOMO->L+2 (88%) |

**Table S3:** Mulliken charge distribution of Asperyellone

| Atoms | Mulliken | Natural charge |
|---|---|---|
| C 1 | -0.06432 | -0.18776 |
| C2 | -0.09491 | -0.07699 |
| C3 | -0.0328 | -0.18644 |
| C4 | -0.0978 | -0.18995 |
| C5 | -0.0803 | -0.19151 |
| C6 | -0.09624 | -0.19332 |
| H7 | 0.09304 | 0.2053 |
| H8 | 0.097822 | 0.20558 |
| H9 | 0.097983 | 0.20583 |
| H10 | 0.099723 | 0.20537 |
| H11 | 0.098782 | 0.20584 |
| C12 | -0.07716 | -0.09802 |
| H13 | 0.130635 | 0.17269 |
| C14 | -0.17595 | -0.21204 |
| H15 | 0.157256 | 0.18399 |
| C16 | -0.12018 | -0.16818 |
| H17 | 0.118117 | 0.17546 |
| C18 | -0.12919 | -0.19861 |
| H19 | 0.163048 | 0.23192 |
| C20 | -0.12805 | -0.20772 |
| H21 | 0.122223 | 0.18017 |
| C22 | -0.05704 | 0.03565 |
| C23 | -0.29352 | -0.60869 |
| H24 | 0.115798 | 0.19861 |
| H25 | 0.12334 | 0.20501 |
| H26 | 0.129665 | 0.19945 |
| C27 | -0.1208 | -0.13899 |
| H28 | 0.125636 | 0.16659 |
| C29 | -0.14318 | -0.20664 |
| H30 | 0.139233 | 0.17843 |
| C31 | -0.12393 | -0.12954 |
| H32 | 0.157914 | 0.19386 |
| C33 | -0.15113 | -0.2446 |
| H34 | 0.122479 | 0.16242 |
| C35 | 0.163503 | 0.47993 |
| C36 | -0.22876 | -0.43049 |
| H37 | 0.122946 | 0.20023 |
| H38 | 0.149416 | 0.21122 |
| O39 | -0.31827 | -0.52928 |
| C40 | -0.32666 | -0.57085 |
| H41 | 0.109839 | 0.1942 |
| H42 | 0.112019 | 0.18715 |
| H43 | 0.10977 | 0.1847 |

206 **Table S4:** Energies associated with delocalization of electron density using the second order
207 Perturbation theory
208

| Donar(i) | Type | ED/e | Acceptor(j) | Type | ED/e | E(2)[a] (KJ mol$^{-1}$) | E(j)-E(i)[b] (a.u) | F(i,j)[c] (a.u) |
|---|---|---|---|---|---|---|---|---|
| C1-C2 | Π | 1.60997 | C5-C6 | π* | 0.37126 | 4.37 | 1.28 | 0.067 |
| C1-C6 | Σ | 1.97749 | C2-C12 | σ* | 0.02679 | 3.78 | 1.09 | 0.057 |
| C2-C3 | Σ | 1.97892 | C1-C2 | σ* | 0.02506 | 4.35 | 1.28 | 0.067 |
| C2-C12 | Σ | 1.97292 | C1-C6 | σ* | 0.01620 | 2.69 | 1.19 | 0.050 |
| C3-C4 | Π | 1.67496 | C1-C2 | π* | 0.37126 | 20.91 | 0.29 | 0.070 |
| C4-C5 | Σ | 1.97913 | C3-C4 | σ* | 0.01661 | 3.01 | 1.27 | 0.055 |
| C5-C6 | Π | 1.65203 | C5-C2 | π* | 0.32714 | 20.00 | 0.29 | 0.068 |
| C12-C14 | Π | 1.80083 | C16-C18 | π* | 0.25257 | 17.08 | 0.26 | 0.060 |
| C14-C16 | Σ | 1.96186 | C12-C14 | π* | 0.25635 | 5.80 | 0.52 | 0.052 |
| C16-C18 | Σ | 1.97975 | C12-C14 | σ* | 0.25635 | 1.99 | 1.09 | 0.042 |
| C16-C18 | Π | 1.69737 | C12-C14 | π* | 0.25635 | 14.89 | 0.20 | 0.049 |
| C18-C20 | Σ | 1.96022 | C22-C27 | σ* | 0.03048 | 5.44 | 0.96 | 0.065 |
| C20-C22 | Π | 1.75211 | C16-C18 | π* | 0.25257 | 14.84 | 0.26 | 0.056 |
| C22-C27 | Σ | 1.95882 | C18-C20 | σ* | 0.03174 | 5.60 | 0.96 | 0.066 |
| C27-C29 | Π | 1.74143 | C31-C33 | π* | 0.21689 | 16.47 | 0.21 | 0.053 |
| C29-C31 | Σ | 1.97443 | C27-C29 | σ* | 0.02123 | 3.31 | 0.52 | 0.038 |
| C31-C33 | Π | 1.76850 | C35-O39 | π* | 0.17214 | 17.12 | 0.27 | 0.061 |
| C33-C35 | Σ | 1.97449 | C31-C33 | π* | 0.21689 | 3.43 | 0.053 | 0.040 |
| LP(2) | Π | 1.90951 | C33-C35 | π* | | 12.86 | 0.62 | 0.081 |

209
210
211
212
213
214
215
216
217

**Table S5.** Molecular physicochemical descriptors analysis on five ligands using Molinspiration online software tool

| Ligand | Log A | TPSA[b] | Natoms [c] | MW[d] | noN [e] | nOH NH [f] | Nviolations [g] | Nrotb [h] | Volume [i] |
|---|---|---|---|---|---|---|---|---|---|
| Asperyellone | 5.34 | 17.07 | 21 | 278.39 | 1 | 0 | 1 | 7 | 290.04 |
| Asperenone | 5.34 | 17.07 | 21 | 278.39 | 1 | 0 | 1 | 7 | 290.04 |
| Hydroasperyellone | 6.26 | 17.07 | 21 | 288.48 | 1 | 0 | 1 | 12 | 321.00 |
| CHEMBL1715716 | 3.54 | 17.07 | 15 | 198.06 | 1 | 0 | 0 | 4 | 202.15 |
| CHEMBL2152350 | 5.94 | 17.07 | 23 | 306.45 | 1 | 0 | 1 | 5 | 318.44 |

([a]_ Octanol-Water partition coefficient, [b]_ Polar surface area, [c]_ Number of non- hydrogen atoms, [d]_ Molecular weight, [e]- Number of hydrogen bond acceptors [ O and N atoms], [f]_ Number of hydrogen bond donors [ OH and NH groups], [g]_ Number of Rule of 5 violations, [h]_ Number of rotatable bonds & [i]_ Molecular volume).

**Table S6.** Bioactivity score of five ligands using Molinspiration online software tool

| Ligand | GPCR[a] ligand | Ion channel modulator | Kinase inhibitor | Nuclear receptor ligand | Protease inhibitor | Enzyme inhibitor |
|---|---|---|---|---|---|---|
| Asperyellone | 0.04 | 0.06 | -0.26 | 0.58 | -0.18 | 0.44 |
| Asperenone | 0.04 | 0.06 | -0.26 | 0.58 | -0.18 | 0.44 |
| Hydroasperyellone | 0.02 | -0.03 | -0.43 | 0.14 | 0.09 | 0.16 |
| CHEMBL1715716 | -0.46 | -0.27 | -0.87 | -0.30 | -0.60 | 0.04 |
| CHEMBL2152350 | -0.07 | -0.14 | -0.32 | 0.68 | -0.06 | 0.44 |

(GPCR ligand [a]- G protein coupled receptors ligand).

**Table S7.** ADME analysis of five ligands

| Ligand | HIA | | | AS | | BBB | | PPB | CYP2D6 | HT |
|---|---|---|---|---|---|---|---|---|---|---|
| | PSA | ALogP98 | L* | Log(SW) | L** | Log BB | L*** | Predication | | |
| Asperyellone | 17.3 | 5.01 | 0 | -4.99 | 2 | 1.12 | 0 | True | False | False |
| Asperenone | 17.3 | 5.01 | 0 | -4.99 | 2 | 1.12 | 0 | True | False | False |
| Hydroasperyellone | 17.3 | 6.62 | 0 | -5.92 | 2 | 1.62 | 0 | True | False | False |
| CHEMBL1715716 | 17.3 | 3.64 | 0 | -3.96 | 3 | 0.70 | 1 | True | False | False |
| CHEMBL2152350 | 17.3 | 5.95 | 0 | -6.42 | 1 | 1.41 | 0 | True | False | False |

(HIA-Human intestinal absorption; AS- Aqueous solubility; BBB- Blood brain barrier; PPB-Plasma protein binding; CYP2D6- cytochrome P450 2D6; HT-hepatotoxicity; F-False & T-True L-Level; *- (0-good; 1-moderate; 2-poor & 3-very poor);**- (0-extremely low; 1-very low; 2-low; 3-good; 4-optimal; 5-too soluble & 6-warning);***-( 0-very high penetrate; 1-high; 2-medium; 3-low & 4-undefined).

**Table S8.** Toxicity predication analysis of five ligands

| Ligand | AB* | AM** | OI# | SI## | SS♦ | Oral toxicity▲ |
|---|---|---|---|---|---|---|
| Asperyellone | Degradable | Non-mutagen | Non-irritant | Irritant | Sensitizer | 1.09 |
| Asperenone | Degradable | Non-mutagen | Non-irritant | Irritant | Sensitizer | 1.09 |
| Hydroasperyellone | Non-degradable | Non-mutagen | Non-irritant | Irritant | Sensitizer | 7.13 |
| CHEMBL1715716 | Degradable | Non-mutagen | Irritant | Irritant | Sensitizer | 0.93 |
| CHEMBL2152350 | Degradable | Non-mutagen | Non-irritant | Irritant | Sensitizer | 1.24 |

(AB*- Aerobic biodegradability; AM**- Ames mutagenicity; OI#- Ocular irritancy; SI##- Skin irritancy; SS♦- Skin sensitization and Oral toxicity▲- Oral toxicity in rat (LD50 in g/Kg of body weight).

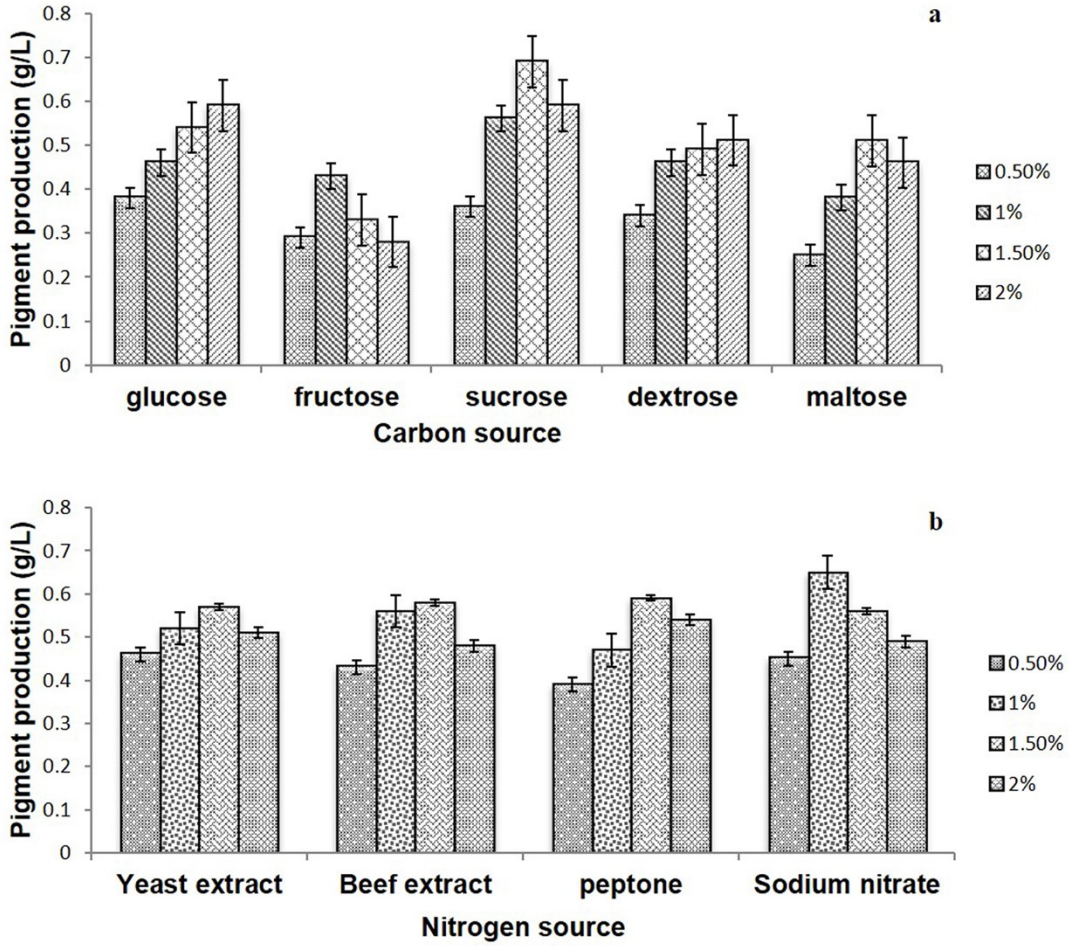



**Fig. S1A.** Optimization of Nutrient components for Asperyellone production by *Apergillus niger* strain AN01. a) Different carbon sources at 0.5%, 1.0%, 1.5% and 2% concentration. b) Different nitrogen sources at 0.5%, 1.0%, 1.5% and 2% concentration. All values are mean ± SD of triplicate samples

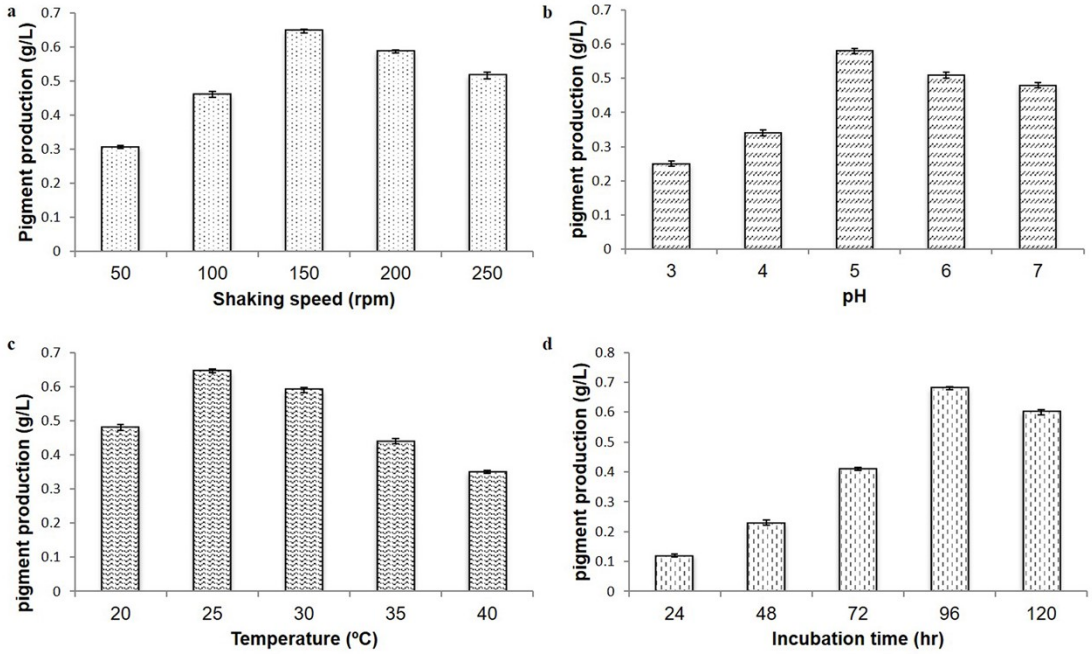
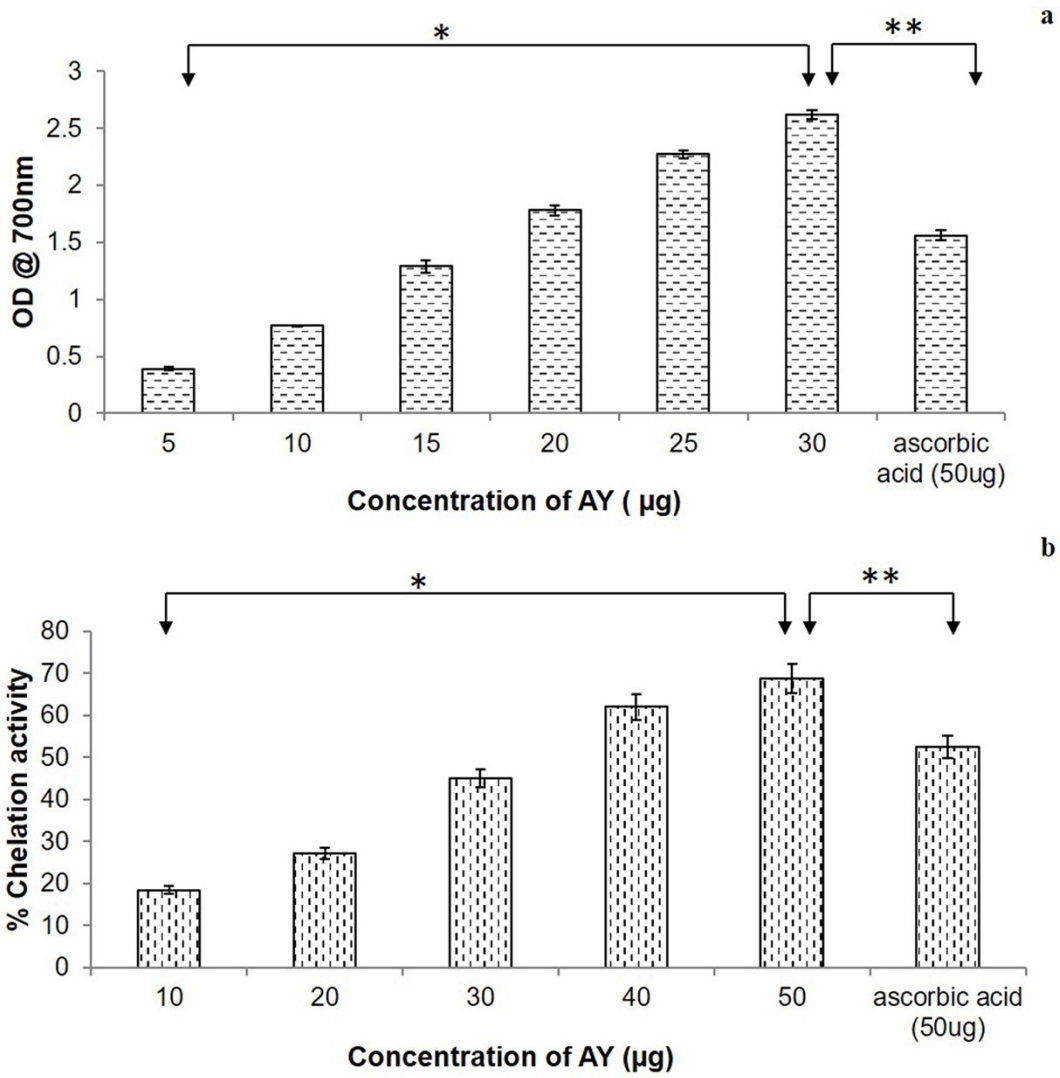
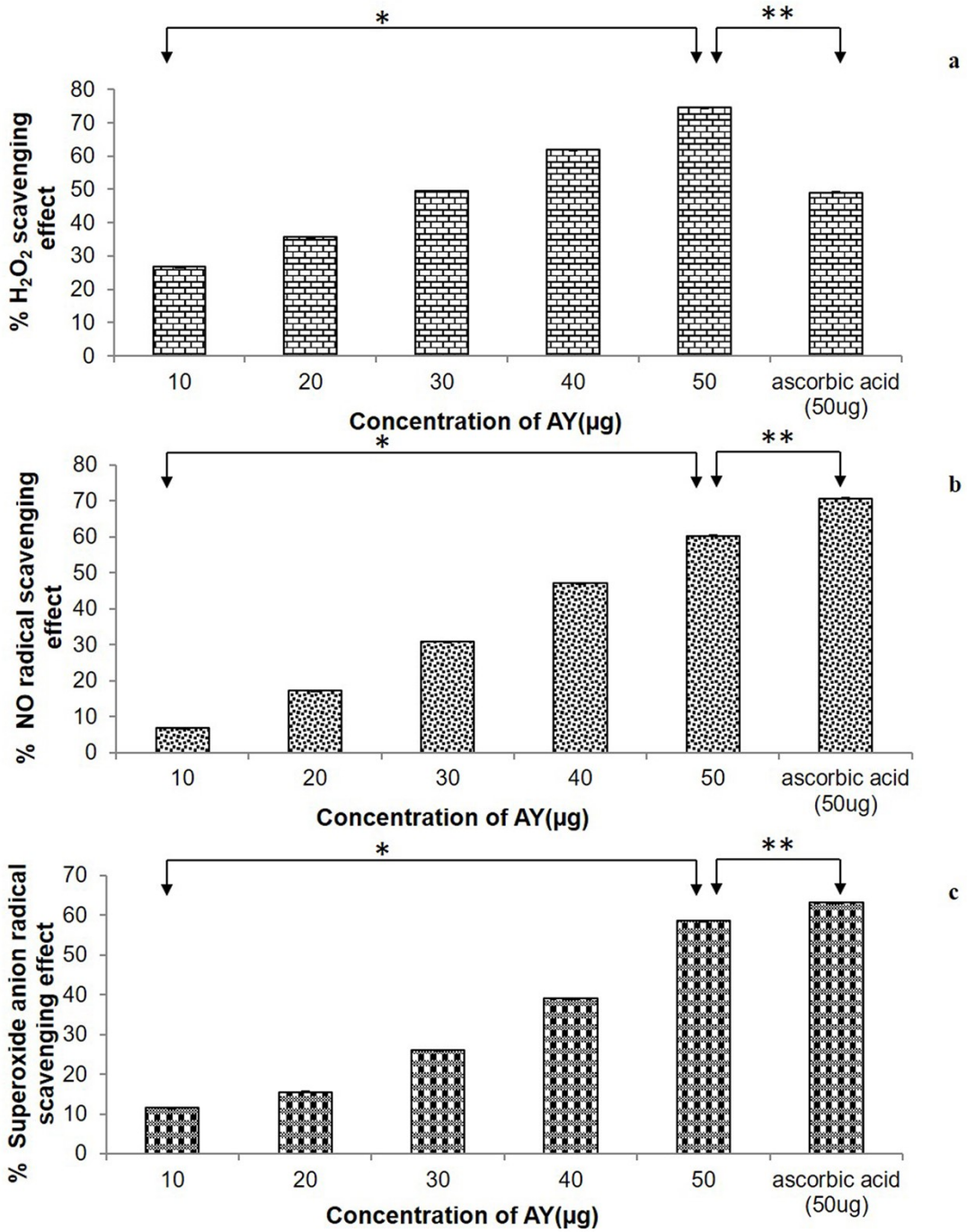



**Fig. S1B.** Optimization of environmental parameters for maximum production of Asperyellone from *Apergillus niger* strain AN01. a) Role of Agitation; b) Role of pH; c) Role of Temperature; d) Role of Incubation period in days. All the values are mean ± SD of triplicates




309
310 **Fig. S2A.** Reducing property of Asperyellone extracted from *Apergillus niger* strain AN01. a) Reducing
311 power assay; b) Iron chelation assay. Increasing concentration displayed significance at *= P<0.05
312 and compared with positive control the significance is at **= P<0.01. All the values are mean±SD
313 of three replicates

314




315
316 **Fig. S2B.** Antioxidant property of Asperyellone extracted from *Apergillus niger* strain AN01. a)
317 Hydrogen peroxide scavenging assay; b) Nitric oxide scavenging assay; c) Superoxide anion
318 scavenging assay. Increasing concentration displayed significance at *= P<0.05 and compared with
319 positive control the significance is at **= P<0.01. All the values are mean±SD of three replicates

320

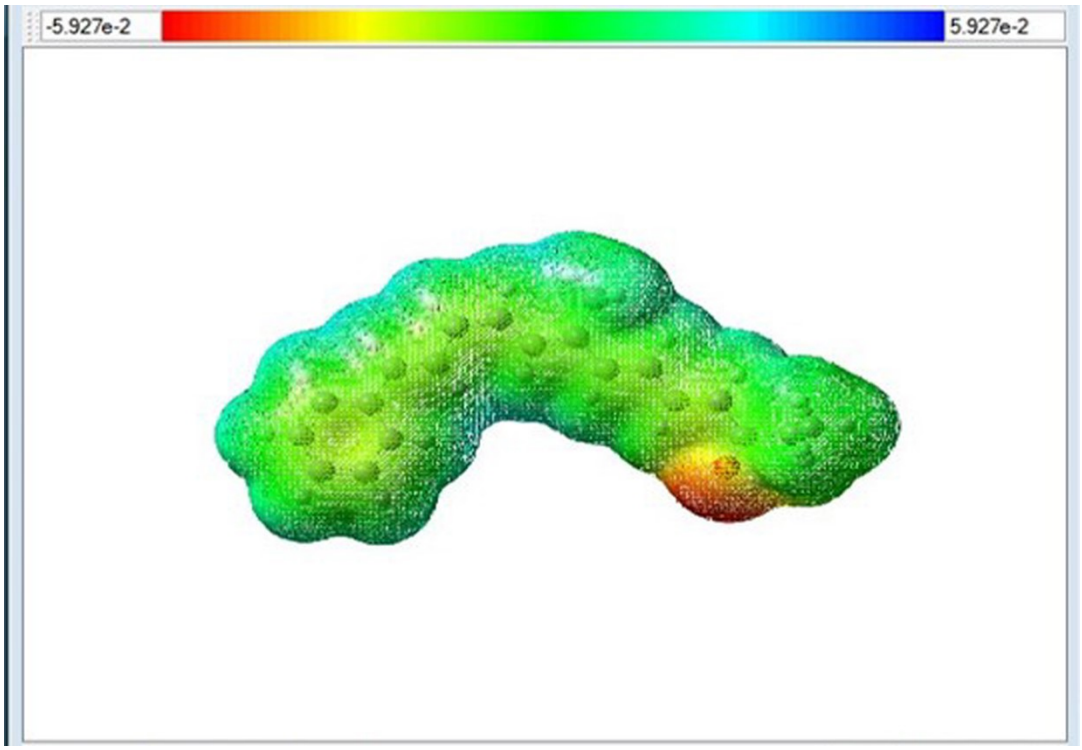



**Fig. S3.** Molecular electrostatic potential map of Asperyellone