**Supplementary materials**

**Chemically characterised *Artemisia nilagirica* (Clarke) Pamp*.* essential oil as a safe plant based preservative and shelf life enhancer of millets against fungal and aflatoxin contamination and lipid peroxidation**

Running head: *Artemisia nilagirica* essential oil as food preservative

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**Section S2. Materials and methods**

*2.1. Chemicals*

Acetone, chloroform, methanol, anhydrous sodium sulphate, Tween-20, sodium hypochlorite, toluene, isoamyl alcohol, sodium chloride, potassium hydroxide, absolute ethanol, n-heptane, paraformaldehyde, glutaraldehyde, osmium tetraoxide, acetone, uranyl acetate, Folin–Ciocalteu reagent, potato dextrose agar (PDA) medium (Potato, 200 g; Dextrose, 20 g; Agar, 15g and double distilled water 1000 mL, pH 5.6±0.2), Silica gel (13% CaSO4.1/2H2O as binder), SMKY medium (Sucrose, 200 g; MgSO4.7H2O, 0.5 g; KNO3, 0.3 g and yeast extract, 7 g; 1000 mL distilled water), 2, 2-azino-bis-3-ethylbenzothizoline-6- sulphonic acid (ABTS•+) and 2,2-diphenyl-1-picrylhydrazil (DPPH•) were procured from HiMedia Laboratories Pvt. Ltd., Mumbai, India.

*2.2. Moisture content and pH of millet samples*

*Sorghum bicolor*(L.) Moench (jowar) and *Eleusine coracana*(L.) Gaertn. (Ragi) seed samples were procured from local market of Varanasi, Uttar Pradesh (India) and Mysore, Karnataka (India), respectively and kept in sterilized polybags to observe the microbial contamination. For assessment of moisture content, 10 g of each sample was dried at 100°C till their weight become constant and this was taken as dry weight. The moisture content was calculated from the following formula

% moisture content=

For pH measurement, 1:10 (sample: distilled water) suspension of each sample was prepared by mixing 10 g powder of each sample in 100 mL of distilled water followed by stirring for 1h. The pH of the suspension was measured using pH meter (Mandeel 2005).

*2.3. Mycoflora analysis of millet sample*

Mycoflora analysis of selected millet samples was carried out following Aziz et al. (1998). After surface sterilization, 10 g of each powdered sample was separately homogenized in 90 mL sterile distilled water in Erlenmeyer flask (250 mL) on electronic shaker at constant speed for 15 min. The sample water suspension was allowed to stand for 10 min. Four fold (10-4) serial dilutions were prepared and 1 mL aliquot of each dilution was aseptically plated and evenly distributed with the help of sterilized L-shaped glass spreader on freshly prepared PDA medium (Fraedrich and Miller 1995). The plates were incubated at 27 ± 2°C for 7 days and inspected daily. Counting and identification of fungal species was done by cultural and morphological characteristics following Raper and Fennel (1977) for the genus *Aspergillus*, Pitt (1979) for the genus *Penicillium* and Domsch et al. (1980) for other molds. The percent relative density and their occurrence frequency of different fungi in each sample were calculated following Singh et al. (2008). The cultures of fungal isolates were maintained on PDA media for further study.

Relative density (%) =

Occurrence frequency (%) =

*2.4. Testing of aflatoxigenicity and selection of most toxigenic strain of Aspergillus flavus*

Aflatoxigenicity of randomly selected *A. flavus* isolates from Ragi and Jowar during mycoflora analysis was assessed in SMKY medium (sucrose, 200 g; MgSO4·7H2O, 0.5 g; KNO3, 0.3 g; yeast extract, 7.0 g; distilled water, 1000 mL) following Kumar et al. (2010). 25 mL SMKY medium was inoculated in 100 mL Erlenmeyer flask separately with 50 µL spore suspension (106 spores per mL) of 7 day old culture of the *A. flavus* isolated from Ragi and Jowar samples. The flasks were kept in a BOD incubator for 10 days at 27 ± 2°C. Thereafter, the content of each flask was filtered by Whatman No. 1, and the biomass was measured after drying the mycelium at 100°C. The filtrate was extracted with 20 mL chloroform in a separating funnel. The separated chloroform extract was evaporated till dryness on water bath at 70°C. The residue left after evaporation was dissolved in 1 mL chloroform and 50 µL of it, was loaded on 20 ×20 cm2 TLC plate (silica gel). The plate was developed in toluene/isoamyl alcohol/methanol (90:32:2; v/v/v) solvent system. The intensity of AFB1 was observed under UV trans-illuminator at an excitation wavelength 360 nm. The blue spots of AFB1 on TLC plate were scrapped out and dissolved in 5 mL methanol, stirred and centrifuged at 3000 rpm for 5 min. The OD360 of supernatant was recorded and the AFB1 content was quantified by following formula:

AFB1 content (µg mL-1) =

Where D, absorbance; M, molecular weight of AFB1 (312); E, molar extinction coefficient of AFB1 (21800 mol-1 cm-1); L, path length (1 cm) (Kedia et al. 2015).

*2.5. Procurement of Artemisia nilagirica and extraction of essential oil*

Aerial parts of *Artemisia nilagirica* (Clarke) Pamp. were collected from the forests of Upper Shillong, East Khasi Hills District, Meghalaya, India. The plant was identified by Prof. N. K. Dubey and the voucher specimen (Voucher specimen No. LHP-AN-AST-06/14) was deposited in Laboratory of Herbal Pesticides, Centre of advanced study in Botany, Banaras Hindu University, U.P., Varanasi, India. The fresh aerial parts of the plant were washed thoroughly by doubled distilled water and subjected to hydro distillation using Clevenger’s apparatus (Clevenger 1928; Prasad et al. 2009) for 5 h. The volatile fraction was separated in form of essential oil (EO) and water traces were removed by adding anhydrous sodium sulphate. The isolated EO was stored in airtight sealed sterile glass vials covered with aluminium foil and kept at 4°C for further testing.

*2.6. Chemical characterization of ANEO*

*2.6.1. Determination of total phenolic content of ANEO*

Total phenolic content (TPC) of ANEO oil was determined spectrophotometrically using the Folin–Ciocalteu reagent according to the method of Gholivand et al. (2010). A solution containing 1000 µg ANEO (thoroughly mixed in 0.1 mL DMSO) was prepared by mixing 46 mL of sterilized distilled water in a volumetric flask and 1 mL Folin–Ciocalteu reagent. The mixture was thoroughly mixed by shaking on electronic shaker and allowed to react for 3 min, thereafter, 3 mL aqueous solution of 2% sodium carbonate (Na2CO3) was added to it. Thereafter, it was left for 2 h of incubation period for the proper reaction at room temperature (25 ± 2°C). At the end of incubation period, the absorbance of each mixture was measured at 760 nm. The same procedure was applied to the standard solutions of gallic acid (0–1,000 µg/ 0.1 mL) and a standard curve was prepared. TPCof the ANEO were obtained by putting the absorbance value at 760 nm to standard curve and equation expressed as mg gallic acid equivalent/mg of the oil.

Absorbance = 0.0012 × gallic acid (μg) + 0.024

*2.6.2. GC and GC-MS analysis of ANEO*

The ANEO was subjected to GC-MS analysis through PerkinElmer Turbomass GC-MS. The GC column was EQUITY-5 (60 m x 0.32 mm x 0.25 µm) fused silica capillary column. The GC conditions were: injection temperature, 250°C; column temperature, at 70°C (isothermal) for 2 min, then programmed to 250°C at 37°C/min and held at this temperature for 10 min; ion source temperature, 250°C. Helium was the carrier gas. The effluent of the GC column was introduced directly into the source of MS and spectra obtained in the EI mode with 70 eV ionization energy. The sector mass analyzer was set to scan from 40 to 500 amu for 2 second. The identification of individual compounds was based on their retention times relative to those of authentic samples and matching their spectral peaks available with Wiley, NIST and NBS mass spectral libraries or with the published data (Adams 2007).

*2.7. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of ANEO*

The antifungal efficacy of the ANEO against toxigenic strain *A. flavus*[LHP(R)-5] was measured in terms of MICand MFCthrough poisoned food technique following Mishra et al. (2013) with slight modifications. Requisite amounts the ANEO were dissolved separately in Petri plates containing 0.5 mL Tween-20 (for homogenization), and 9.5 mL potato dextrose agar (PDA) medium was poured in plates to obtain different concentrations varying from 0.1 to 1.4 µL mL-1. The plates containing Tween-20 (0.5 mL) and PDA (9.5 mL) medium only served as control. A fungal disc (5.0 mm diameter) of *A. flavus*[LHP(R)-5] taken from 7 days old culture was aseptically inoculated to prepared Petri dishes which were then incubated at 27±2 °C for 7 days. The percent inhibition of fungal growth was calculated by the following formula:

Percent mycelial inhibition =

Where, Dc= average diameter of fungal colonies in control sets and Dt = average diameter of fungal colonies in treatment sets.

The minimum concentration of ANEO at which growth of *A. flavus* was completely absent, considered as the MIC. For assessment of MFC, the inhibited fungal discs of oil treated sets were re-inoculated on fresh medium and the lowest concentration of ANEO at which there was no revival of the fungal growth was considered as the MFC (Prakash et al. 2012).

*2.8. Determination of minimum aflatoxin B1 inhibitory concentration (MAIC) of ANEO*

The minimum aflatoxin B1 inhibitory concentration (MAIC) of ANEO was recorded against the toxigenic strain *A. flavus*[LHP(R)-5] following Shukla et al. (2008). Requisite amounts of the EO were dissolved separately in 0.5 mL Tween-20 and 24.5 mL SMKY broth medium in 100 mL conical flasks so as to get different concentrations of ANEO (0.1 µL mL–1 – 1.4 µL mL–1). Conical flasks containing only Tween-20 (0.5 mL) and SMKY medium (24.5 mL) served as controls. The flasks were then inoculated with 50 µL of spore suspension (approx.106 spores mL–1) of the toxigenic strain *A. flavus*[LHP(R)-5] and incubated at 28 ± 2°C for 10 days. The AFB1 content of each treatment set was determined by the method earlier mentioned in section 2.4. The minimum concentration caused complete inhibition of AFB1 production was designated as MAIC. The dry weight of each set was also measured.

*2.9. Fungitoxic spectrum of ANEO*

The toxicity of the ANEO was assessed against thirteen food borne fungal species isolated, from millet samples during mycoflora analysis, was evaluated by poisoned food technique (Dwivedy et al. 2016). The ANEO at its MIC (1.4 µL mL–1) was added separately to Petri plates containing 0.5 mL Tween-20 (5%) and 9.5 mL PDA medium. A 5 mm disc from a seven day old colony from each fungus (*Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus sydowii*, *Aspergillus minutus*, *Penicillium italicum*, *Penicillium purpurogenum*, *Alternaria alternata*, *Cheatomium spirale*, *Curvularia lunata*, *Rhizopus stolonifer*, *Mucor* sp. and Mycelia sterilia) was separately placed on the centre of the prepared Petri plates. Plates containing fungal inoculum only in Tween-20 (0.5 mL) and 9.5 mL PDA served as control. The plates of both treatment and control sets were incubated at 27±2°C for seven days.

The percent inhibition of fungal growth was calculated by the formula mentioned in section 2.7.

*2.10. Mode of action of ANEO against aflatoxigenic strain of A. flavus*

*2.10.1. Electron microscopic observations*

5 days old culture of *A. flavus*[LHP(R)-5] grown on PDA plates was exposed to 1.4 and 2.8 µL mL–1 of ANEO while control sets were without ANEO. After 7 days of exposure, 2×2 mm segments from the margin were taken and placed in test tube at 4°C containing 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer and fixed overnight. The fixed samples were washed thrice with the same buffer and then post fixed in 1% osmium tetraoxide for 1 h at 4°C. The post fixed samples were dehydrated for a period of 20 min in a series of graded acetone up to absolute in each dilution. For SEM, the samples were finally sputter coated with platinum and observed under SEM. For TEM, the samples were washed with toluene and then polymerized in araldite CY212 (TAAB, UK) overnight in an oven at 50°C. Ultrathin sections (60–70 nm) of the specimen blocks were cut with an ultra-microtome (Leica EMUC6) and placed over copper grids (300 mesh). The sections were stained first with 12.5% alcoholic uranyl acetate for 20 min and then with lead–citrate. After rinsing with double distilled water for 1 min, the samples were dried and observed under TEM at an operating voltage 200 kV. Images were digitally taken by a CCD camera (Megaview III, Fei Company) using iTEM software (Sift Imaging System, Münster, Germany) attached to the microscope (Kedia et al. 2015).

*2.11.2. Effect of ANEO on ergosterol content in plasma membrane of A. flavus[LHP(R)-5]*

Presence of ergosterol content in the plasma membrane of *A. flavus*[LHP(R)-5] was assessed following Tian et al. (2012) with slight modifications. A 100 μL aliquot of spore suspension of *A. flavus* strain[LHP(R)-5] (106 spores mL–1) in 0.1% Tween-20 was inoculated in SMKY medium containing 0.0, 0.25, 0.50, 0.75 and 1.0 µL mL–1 of ANEO. After 4 days of incubation at 27 ± 2°C, mycelia were harvested and washed thrice with doubled distilled water. The net wet weight of the cell pellet was measured. Five mL of 25% alcoholic potassium hydroxide solution (25 g KOH and 35 mL sterile distilled water, brought to 100 mL with 100% ethanol) was added to each cell pellet and vortex mixed for 2 min, followed by incubation at 85°C in a water bath for 4 h. Sterols were then extracted from each sample by adding a mixture of 2 mL sterile distilled water and 5 mL n-heptane followed by sufficient vortexing for 2 min. The layers were allowed to separate for 1 h at room temperature, and the n-heptane layer was separated and scanned by Uv-Vis spectrophotometer between 230 and 300 nm. The presence of ergosterol and the late sterol intermediate 24(28) dehydroergosterol in the n-heptane layer resulted in a characteristic four peaked curve. The absence of ergosterol in samples was indicated by a flat line. The ergosterol amount was calculated as a percentage of the wet weight of the cells as follows:

% ergosterol + % 24(28) dehydroergosterol= (A282/290)/pellet weight; % 24(28) dehydroergosterol= (A230/518)/pellet weight; and % ergosterol = (% ergosterol + %24(28) dehydroergosterol)- % 24(28) dehydroergosterol.

Where, 290 and 518 are the E values (% cm-1) determined for crystalline ergosterol and 24(28) dehydroergosterol, respectively, and pellet weight is the net wet weight (g).

*2.11.3 Measurement of ions leakage from the cells of A. flavus[LHP(R)-5] treated with ANEO*

The culture of *A. flavus*[LHP(R)-5] was grown in SMKY medium for 5 days. The biomass was filtered, washed thrice with sterile doubled distilled water and suspended in 20 mL 0.85% saline solution and fumigated with MIC and MFC concentrations of test EO for 12 h while control sets received no EO. The biomass were filtered again and the supernatant was analysed using atomic absorption spectrometry (PerkinElmer, AAnalyst 800, USA) for Ca2+, K+ and Mg2+ ions (Helal et al. 2007; Kedia et al. 2015).

*2.12. Antioxidant activity of ANEO*

*2.12.1. DPPH• (2, 2-Diphenyl-1-picrylhydrazil) assay*

Free radical scavenging activity of the ANEO was measured by recording the extent of bleaching of a DPPH• solution from purple to yellow following Prakash et al. (2012). Different concentrations (0.25 to 4.0 µL mL–1) of the ANEO were added to 0.004% DPPH• solution in methanol (5 mL). After 30 min incubation at room temperature (27 ± 2°C), the absorbance was measured against a blank at 517 nm using a spectrophotometer. The antioxidant activity was measured through scavenging of DPPH• free radical with reduction in absorbance of the sample. The IC50 of ANEO, which represented the concentration that caused 50% neutralization of DPPH• radicals, was measured from the graph plotting percentage inhibition against concentration.

Inhibition% = (Ablank−Asample/Ablank) ×100

Where, Ablank is the absorbance of the control (without test material) and Asample is the absorbance of the test material.

*2.12.2. ABTS•+ (2, 2-azino-bis-3-ethylbenzothizoline-6-sulphonic acid) assay*

ABTS•+ scavenging activity of ANEO was also measured following the previously reported procedures with slight modifications (Re et al. 1999; Kiran et al. 2016). The ABTS•·+ radical reaction mixture was prepared by reacting 140 mM potassium per sulphate with 7 mM ABTS•+ as stock solution and kept in the dark at room temperature (27 ± 2°C) for 12 h. Thereafter, ABTS•+ solution was diluted with phosphate buffer saline (pH = 7.4) to obtain a solution with absorbance of 0.70 ± 0.05 at 734 nm. Appropriate concentration (0.5 to 2.5 µL mL–1) of ANEO was prepared with ethanol then homogenised with 3 mL of the ABTS•+ solution and absorbance at 734 nm was measured after 6 min of reaction at room temperature. Previously reported formula (section 2.12.1) was used for the calculation of percent inhibition.

*2.13. In situ efficacy of ANEO against fungal deterioration of Ragi during storage*

*In situ* efficacy of ANEO against fungal deterioration of Ragi samples was performed in airtight containers during storage following method of Kedia et al. (2014). In one set (uninoculated treatment), requisite amount of ANEO, soaked in a cotton swab was introduced in airtight closed plastic container, containing 500 g of Ragi seeds (var. K2, moisture content 11.35±0.47) to achieve a concentration of 1.4 μl mL–1 (MIC) in air. In another set (inoculated treatment), the Ragi samples, were inoculated with 1 mL spore suspension of *A. flavus*[LHP(R)-5] prior to treatment with the ANEO. Two control sets were also maintained, one by inoculating with spore suspension and one without inoculation. After 12 months of storage under laboratory conditions (temperature 10–46°C and RH 30–90%), the mycoflora analysis of Ragi samples of both treatment and control sets were performed by the method earlier mentioned in the section 2.3. The percent protection in the uninoculated and inoculated treatments sets were calculated by following formula.

% protection =

Where, Dc = % occurrence of total fungi in control set and Dt = % occurrence of total fungi in treatment set.

*2.14. Safety profile of ANEO*

The safety profile of the ANEO was determined on male mice (*Mus musculus* L., average weight 30 g, age 3 months) by recording their LD50 values which represent the lethal dose of EO per unit weight for killing of 50% population of test animals (Singh et al. 2009). A stock solution of Tween-20 and distilled water (1:1) was prepared. Different doses of ANEO from 0.05 mL to 0.4 mL were orally administered to each group of animal (10 mice) separately with 0.5 mL stock solution. In control set, equal dose of Tween-20 and distilled water was given. After 4 h, the mortality of the test animals was recorded and LD50 of ANEO was calculated by probit analysis (Finney 1971).

*2.15. Phytotoxicity assessment of ANEO*

Phytotoxic assessment of ANEO was performed in terms of germination of Ragi millet seeds with respect to the control sets following the ISTA guideline (2013). The millet seeds of control and treatment sets (1.4 µL mL–1) during *in situ* experiment (section 2.14), were tested for germination assay. After one year exposure to ANEO, 20 randomly selected seeds from each set were placed in Petri plates containing moistened blotting paper. Thereafter, Petri plates were sealed with paraffin film and placed in incubator for germination. The percent germination and viability of seeds of control and treated sets were recorded at regular interval of time up to seven days.

*2.16. Statistical analysis*

Experiments were performed in triplicates and data were presented as mean ± standard error subjected to one way analysis of variance (ANOVA). Means are separated by Tukey's multiple range tests when ANOVA was significant (p < 0.05). The analysis of data was done with the help of SPSS program version 16.0 (IBM Corporation).

**Table S1.** Chemical profile of *A. nilagirica* essential oil

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| S. No. | Compound | Retention time (min) | Retention Index | % |
| 1 | (3E)-2,5,5-Trimethyl-1,3,6-heptatriene | 6.171 | 918.27 | 0.025 |
| 2 | Delta.3-carene | 6.850 | 951.60 | 0.016 |
| 3 | Camphene | 7.180 | 965.90 | 0.113 |
| 4 | 1-Octen-3-ol | 7.328 | 970.06 | 1.896 |
| 5 | Sabinene | 7.469 | 983.46 | 0.375 |
| 6 | 2-Beta.-Pinene | 7.550 | 993.53 | 2.983 |
| 7 | 2,7-Dimethyl-2,6-octadien-4-ol | 7.994 | 1006.09 | 0.073 |
| 8 | Alphaterpinene | 8.310 | 1017.87 | 1.252 |
| 9 | Benzene,methyl(1-methylethyl) | 8.492 | 1025.36 | 2.967 |
| 10 | Eucalyptol | 8.748 | 1029.18 | 0.248 |
| 11 | 1,5- Heptadiene-4-one,3,3,6-trimethyl- | 9.037 | 1056.18 | 32.722 |
| 12 | Gamma-terpinene | 9.219 | 1061.16 | 0.314 |
| 13 | Artemisia alcohol | 9.616 | 1079.23 | 13.397 |
| 14 | Z-E epoxy-ocimene | 9.898 | 1086.96 | 0.321 |
| 15 | 2,6- dimethyl-3,5,7-octatriene-2-0L,z,z- | 10.033 | 1093.14 | 0.838 |
| 16 | Linalool | 10.127 | 1095.99 | 1.288 |
| 17 | 1,7-octadien-3-one,2-methyl-6-methylene- | 11.238 | 1105.22 | 0.030 |
| 18 | 1,5,7-Octatrien-3-ol,2,6-dimethyl- | 11.743 | 1109.50 | 0.024 |
| 19 | Camphor | 11.978 | 1114.08 | 0.097 |
| 20 | 1-Methylethenyl | 12.059 | 1115.52 | 0.265 |
| 21 | Benzenepropanal | 12.214 | 1118.66 | 0.129 |
| 22 | Endo-borneol | 12.691 | 1133.38 | 0.833 |
| 23 | 3-Cyclohexen-1-ol,4-methyl-1-(1-methylethyl)- | 12.886 | 1137.15 | 1.221 |
| 24 | Methylsalicylate | 13.270 | 1150.55 | 1.108 |
| 25 | 1,5-Heptadien-4-one,3,3,6-trimethyl- | 14.468 | 1153.35 | 0.238 |
| 26 | Alpha -lonone | 14.953 | 1159.84 | 4.553 |
| 27 | 1-Cyclohexene-1-carboxaldehyde,4-(1-methylethyenyl)- | 16.211 | 1231.37 | 2.236 |
| 28 | Bicyclo[2.2.1]heptane-2-ol,1,7,-trimethyl-,acetate ,(1S-endo) | 16.386 | 1236.27 | 0.072 |
| 29 | 1,3-Cyclohexadiene-1-carboxaldehyde,266-trimethyl | 16.635 | 1246.69 | 0.226 |
| 30 | 4-Methyl-7-methylethyl-3,8-dioxatriccyclo[5.1.0(2-4)octane | 17.302 | 1265.77 | 0.535 |
| 31 | Junipene | 19.812 | 1308.97 | 0.039 |
| 32 | Alfa-copaene | 19.994 | 1343.30 | 0.017 |
| 33 | Germacrene-D | 20.391 | 1350.00 | 0.048 |
| 34 | Caryophyllene | 21.771 | 1369.56 | 0.275 |
| 35 | Trans-beta-farnesene | 22.282 | 1383.79 | 0.401 |
| 37 | Beta -himachalene | 23.668 | 1412.34 | 1.230 |
| 38 | Germacrene-D | 24.079 | 1428.83 | 0.032 |
| 39 | Germacrene-D | 24.523 | 1436.63 | 0.325 |
| 40 | 1-Isopropyl-4-methyl-7-methylene-1,2,3,4,4A,5,6,7-octahydronap | 24.543 | 1446.14 | 0.299 |
| 41 | Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl) | 25.229 | 1451.11 | 0.144 |
| 42 | Delta-cadinene | 25.364 | 1473.56 | 0.230 |
| 43 | 1H-cycloprop[e]azulen-7-ol,decahydro-1,1,7-trimethyl-4-methyle | 27.767 | 1516.62 | 1.358 |
| 44 | (-)-Caryophyllene oxide | 28.043 | 1574.51 | 1.570 |
| 45 | Longifolene | 29.025 | 1557.40 | 0.456 |
| 46 | a-Copaene-8a-ol | 29.476 | 1566.71 | 0.116 |
| 47 | 4-Epi-cubedol | 29.597 | 1579.43 | 0.542 |
| 48 | Agaruspirol | 29.758 | 1574.51 | 1.570 |
| 49 | Isoledene | 30.081 | 1590.02 | 0.091 |
| 50 | Torreyol | 30.155 | 1579.81 | 0.771 |
| 51 | Torreyol | 30.249 | 1599.91 | 0.266 |
| 52 | t-Muurolol | 30.586 | 1603.23 | 0.086 |
| 53 | 2-Naphthalenemethanol, 1,2,3,4,4A,5,6,8a-octahydro-alpha.,alpha | 30.694 | 1606.18 | 0.090 |
| 54 | Selina-6-en-4-ol | 30.822 | 1620.46 | 0.811 |
| 55 | Alpha-bisabolol | 31.548 | 1676.31 | 1.897 |

**Note:** The components were determined by MS data and also by comparing with the retention times of authentic compounds (internal standards)

**Picture2.tif**

**Figure S1.** GC-MS chromatogram of ANEO



**Figure S2.** Fungitoxic spectrum of ANEO at 1.4 µL mL-1 concentration against 13 storage fungi of millets