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Toxic, but beneficial compounds from endophytic fungi of *Carica papaya*

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Abstract

Fungi remain a promising source of novel biologically active compounds with potentials in drug discovery and development. This study was aimed at investigating the secondary metabolites from endophytic *Fusarium equiseti* and *Epicoccum sorghinum* associated with leaves of *Carica papaya* collected from Agulu, Anambra State, Nigeria. Isolation of the endophytic fungi, taxonomic identification, fermentation, extraction and isolation of fungal secondary metabolites were carried out using standard procedures. Chromatographic separation and spectroscopic analyses of the fungal secondary metabolites yielded three toxigenic compounds - equisetin and its epimer 5'- epiequisetin from *F. equiseti,* and tenuazonic acid from *E. sorghinum.* These compounds are known to possess several beneficial biological properties that can be explored for pharmaceutical, agricultural or industrial purposes.

Keywords: Endophytic fungi, *Carica papaya*, *Fusarium equiseti*, *Epicoccum sorghinum*, secondary metabolites

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Introduction

Carica papaya (Caricaceae) also known as papaya, papaw, or pawpaw is a herbaceous plant indigenous to most tropical regions of the world, including Nigeria (1, 2). Traditionally, the plant is used for the treatment of a wide range of ailments like wounds, ulcers, menstrual irregularities, thermal traumas, dyspepsia, diarrhea, bleeding, haemorrhoids, whooping cough, dysentery, skin diseases and impotence (1-3).

The plant has been reported to show anti-inflammatory (4,5), wound healing (2, 3), anti-fertility (6, 7), antihelmintic (8), anticancer (9, 10), antimicrobial (11-14), anti-hypertensive (15), immunomodulatory (16), antimalarial (17), and diuretic (18, 19) activities.

Fungi are known to be predominantly associated with *C. papaya* diseases and spoilage. These include fungi of the genera *Colletotrichum, Fusarium, Aspergillus, Phoma, Cladosporium, Phomopsis, Penicillium, Rhizopus*, etc (20-24). Notwithstanding their ability to cause diseases, some fungi exist asymptomatically and symbiotically as endophytes in the tissues of the *C. papaya* without causing disease symptoms. Reports have been made of endophytes associated with *C. papaya* with a view of evaluating the potentials of the plant's endophytic populations in bioprospecting for novel bioactive molecules. Bacterial and fungal endophytes from *C. papaya* capable of producing important extracellular enzymes with biotechnological potentials have been reported (25, 26). Secondary metabolites from a fungus isolated from the leaves of *C. papaya* showed excellent cytotoxic activity against mouse lymphoma cells (27). Wang *et al.* (28) reported the isolation several bioactive compounds from an endophytic *Aspergillus aculeatus* associated with *C. papaya*. Some of the isolated compounds, asperdichrome, RF 3192C, secalonic acids D and F, showed significant cytotoxic activities (28).

In this present study, two endophytic fungi were isolated from healthy leaves of *C. papaya*, and the fungi were identified to be toxigenic species in the genera *Fusarium* and *Epicoccum*. The fungi were chemically investigated for their secondary metabolites.

Materials and Methods

Collection of plant materials

Fresh leaves of *C. papaya* were collected from Agulu, Anambra State, Nigeria. The plant material was authenticated and a voucher specimen (PCG474/A/023) deposited in the herbarium of the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Anambra State, Nigeria.

Isolation and identification of endophytic fungi

Isolation of endophytic fungi from the leaves of *C. papaya* was carried out as described by Eze *et al.* (29). The taxonomic identification of the endophytic fungi carried out using DNA amplification and sequencing of the fungal ITS region (30). Two endophytic fungi were isolated and were identified as *Fusarium equiseti* and *Epicoccum sorghinum.* Their DNA sequence data were deposited in the NCBI database (GenBank) with accession numbers KX137847 and MH269246 respectively.

Fermentation, extraction, fractionation and isolation of metabolites

Solid state fermentation was carried out by growing each fungus in 1 L Erlenmeyer flask containing sterile solid rice medium (100 g of rice + 100 mL of distilled water, autoclaved at 121 °C at 15 psi for 1 h) under static conditions at 22 °C for 14 days. After fermentation, the fungal secondary metabolites were extracted with ethyl acetate (EtOAc) and then concentrated under reduced pressure. A weight of 1.5 g of *F. equiseti*'s crude extract (code name: CPL1) was subjected to vacuum liquid chromatography (VLC) on silica gel 60, and stepwise gradient elution of the extract was carried out using dichloromethane:methanol (DCM:MeOH). A dry weight (67.3 mg) of the VLC fraction (DCM 80%:20% MeOH) containing the metabolites of interest was further separated on Sephadex LH-20 with DCM:MeOH (1:1 (v/v)) as the mobile phase. Also, 330 mg of *E. sorghinum*'s crude extract (code name: CP1) was separated on Sephadex LH-20 using DCM:MeOH (1:1 (v/v)) as the mobile phase. The metabolites-containing fractions were further purified using semi-preparative HPLC to isolate the pure compounds. Compounds **1** (4.4 mg) and **2** (1.9 mg) were isolated from *F. equiseti*'s extract; while compound **3** (3.8 mg) was isolated from the extract of *E. sorghinum.* Analytical HPLC was used to evaluate the purity of the isolated compounds.

General procedures

NMR measurements of the isolated compounds were carried out in deuterated chloroform (compounds **1** and **2**) and deuterated methanol (compound **3**) using a Bruker Avance DMX 600 spectrometer (Bruker BioSpin, Germany). The NMR spectra were referenced relative to the residual solvent signals. For mass spectral analysis, LC-MS measurements were carried out using a Finnigan LCQ Deca XP LC-MS System (Thermo Electron, Germany); while HR-ESIMS was measured with a UHR-QTOF maXis 4G (Bruker Daltonik, Germany) mass spectrometer. Analytical HPLC analysis was carried out using a Dionex P580 system coupled to a P580A LPG pump and a photodiode array detector (UVD340s, Dionex Softron, Germany). The HPLC instrument consists of a separation column (125 x 4 mm) prefilled with Eurosphere-10 C18 (Knauer, Germany) with MeOH-H_2O mixtures as the gradient solvent system. Semi-preparative HPLC was performed using a Merck-Hitachi HPLC System comprising of a UV detector (L-7400), pump (L-7100), and a Eurosphere column (100 C18, 300 × 8 mm, Knauer, Germany). Gradient MeOH-H_2O mixtures were used as the mobile phase at a flow rate of 5.0 mL/min. Vacuum liquid and open column chromatography were carried out using Silica gel 60 (70–230 mesh, Merck, Germany) and Sephadex LH-20 (Sigma-Aldrich, Germany) respectively. Pre-coated TLC plates (silica gel 60 F254, 20× 20 cm, 0.25 mm thick, Merck, Germany) were used to monitor fractions under UV detection (Camag UV cabinet, Germany) at 254 and 366 nm. The optical rotation of compounds **1** and **2** were measured in MeOH using a P-2000 polarimeter (Jasco, Germany). Distilled solvents were used for column chromatography and spectral-grade solvents were used for spectroscopic measurements.

Results

Chromatographic separation and spectroscopic analyses of the fungal secondary metabolites resulted in the isolation of three compounds (compounds **1** and **2** from *F. equiseti*; and **3** from *E. sorghinum*). HPLC chromatograms of the fungal crude extracts and UV-spectra of the detected compounds (showing their peaks and retention times (RT)) are presented in **Figs. 1** and **2**. Also, structures of the isolated compounds are shown in **Fig. 3**.

Compound 1

Compound **1** showed UV maxima at 208.6, 234.9 and 297.0 nm. LC-MS analysis revealed pseudomolecular ions at *m/z* 374.1 $(M+H)^+$ and 372.5 $(M-H)^-$ upon positive and negative ionization respectively, thus revealing a molecular weight of 373 g/mol. Analysis of the ^{1}H-NMR data ($CDCl_3$, 600 Hz) suggested the molecular formula $C_{22}H_{31}NO_4$. The optical rotation $[\alpha]_D^{20}[\alpha]_D^{20}$ = -237.6° (*c* 0.2, MeOH) was recorded. Based on the spectral data, the compound was identified as equisetin (IUPAC name: (3E,5S)-3-(((1S,2R,4aS,6R,8aR)-1,6-dimethyl-2-((E)-prop-1-enyl)-4a,5,6,7,8,8a-hexahydro-2H-naphthalen-1-yl)-hydroxymethylidene)-5-(hydroxymethyl)--1-methylpyrrolidine-2,4-dione). This compound was originally described from *Fusarium equiseti* (31-34).

Compound 2

Compound **2** also showed UV maxima at 203.2, 234.4 and 295.5 nm. The LC-MS displayed pseudomolecular ions at *m/z* 374.1 $(M+H)^+$ and 372.5 $(M-H)^-$ upon positive and negative ionization

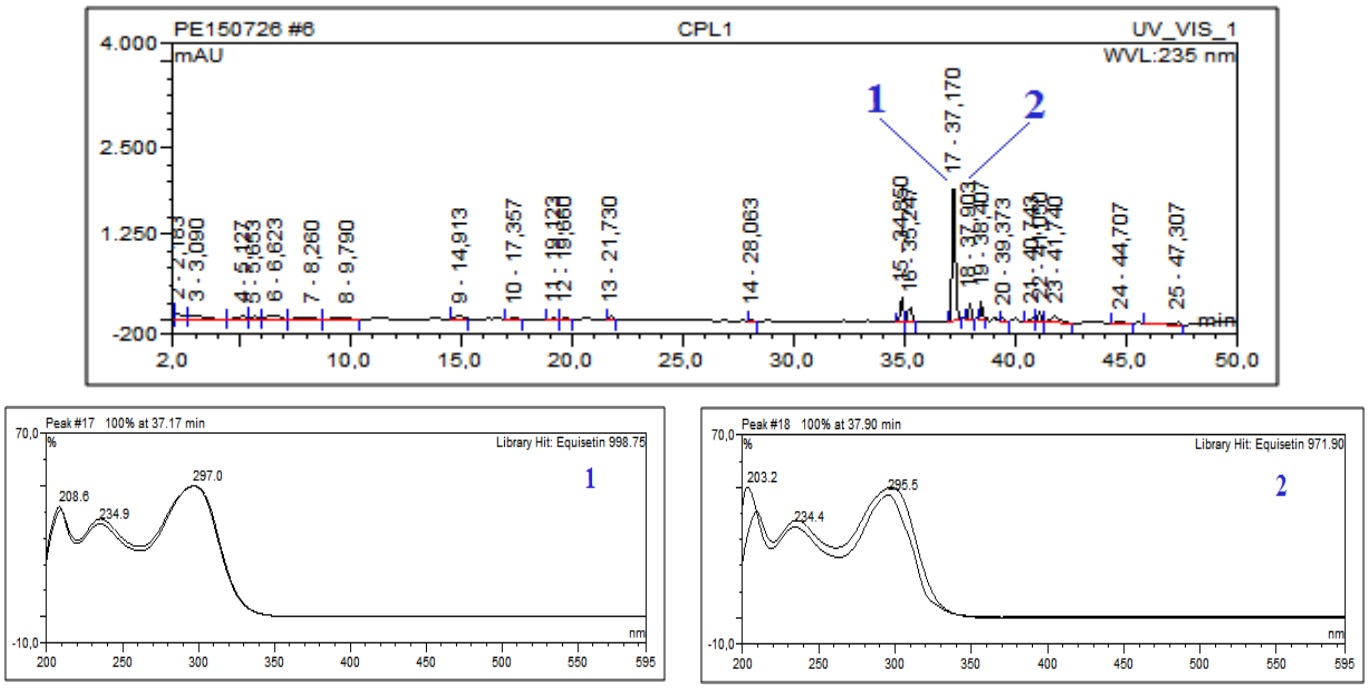


Figure 1. HPLC Chromatogram of *Fusarium equiseti*'s crude extract (CPL1); and UV-spectra of compounds **1** (Peak 17, RT 37.17 min) and **2** (Peak 18, RT 37.90 min).

respectively, thus revealing a molecular weight of 373 g/mol. Interpretation of the ^{1}H-NMR data ($CDCl_3$, 600 Hz) suggested the molecular formula $C_{22}H_{31}NO_4$. The optical rotation $[\alpha]_D^{20}$ $[\alpha]_D^{20}$ = -196.4° (*c* 0.2, MeOH) was recorded. The compound was thus identified as 5'-epi-equisetin, an epimer of equisetin (IUPAC name: (3E,5R)-3-(((1S,2R,4aS,6R,8aR)-1,6-dimethyl-2-((E)-prop-1-enyl)-4a,5,6,7,8,8a-hexahydro-2H-naphthalen-1-yl)-hydroxy-methylene)-5-(hydroxymethyl)-1-methyl-pyrrolidine-2,4-dione). Results of the spectroscopic analysis of compound **2** are confirmed by the report of Phillips *et al.* (33).

Compound 3

Compound **3** displayed UV maxima at 218.0 and 277.3 nm. HR-ESIMS showed pseudomolecular ions at *m/z* 196.3 $(M-H)^-$ upon negative ionization, revealing a molecular weight 197 g/mol Interpretation of the ^{1}H-NMR data (MeOD, 600 Hz)

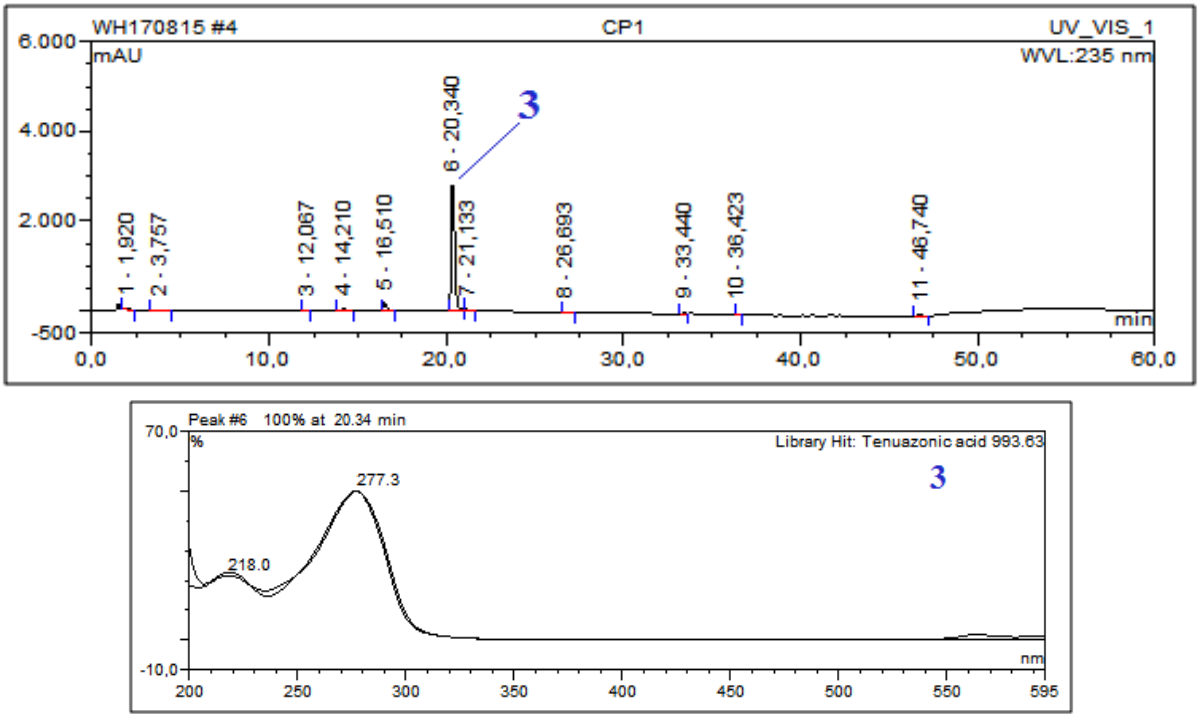
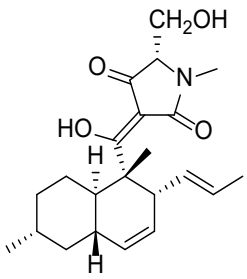
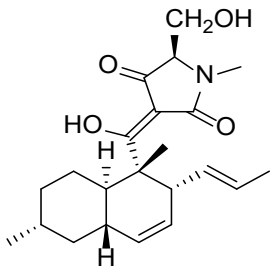


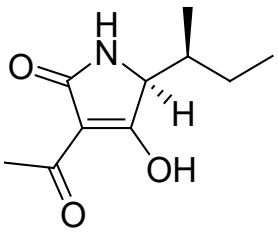
Figure 2. HPLC Chromatogram of *Epicoccum sorghinum*'s crude extract (CP1); and UV-spectrum of compound **3** (Peak 6, RT 20.34 min).



Compound 1
(Equisetin ($C_{22}H_{31}NO_4$. 373 g/mol))



Compound 2
(5′-Epiequisetin ($C_{22}H_{31}NO_4$.373 g/mol))



Compound 3
(Tenuazonic acid ($C_{10}H_{15}NO_3$.197 g/mol))

Figure 3. Structures of isolated compounds.

suggested the molecular formula $C_{10}H_{15}NO_3$. Compound **3** was thus elucidated as a tetramic acid derivative - tenuazonic acid (IUPAC name: (5*S*)-3-acetyl-5-((2*S*)-butan-2-yl)-4-hydroxy-2,5-dihydro-1*H*-pyrrol-2-one). Results of the spectroscopic analysis of the isolated compound are confirmed by the report of Davis *et al.* (35).

Discussion

Fusarium species are important plant pathogens that are widely distributed throughout the world. They can exist as opportunistic colonizers of plants and agricultural commodities, or as saprophytes on debris and plant materials (32,36). Several species of *Fusarium* cause a broad range of plant diseases, including vascular wilt, seedling blight, fruit, root or stem rot, and cereal ear rot. Some *Fusarium* strains can synthesize several mycotoxins like the trichothecenes, zearalenones, and fumonisins. Moniliformin, beauvericin, and fusaproliferin have also been synthesized by the fungal strains (36). Among the endophytes from medicinal plants, many studies have revealed *Fusarium* sp. as the most prevalent species and a potent source of biologically active compounds (37, 38). Many studies have been carried out on metabolites production from several *Fusarium* species especially *F. oxysporum* and *F. solani* (39-42).

Fusarium equiseti (Nectriaceae) (teleomorph: *Gibberella intricans*) is a toxigenic species and a soil inhabitant known to cause disease in several plant species. The fungus can infect seeds, fruits, roots and tubers of several crop plants from diverse climatic regions (43, 44). *F. equiseti* is capable of expressing a vast range of phytotoxic and cytotoxic metabolites (45, 46).

The *Fusarium* toxin equisetin, an N-methylserine-derived acyl tetramic acid, was first isolated in 1974 from the white mold *F. equiseti* (46). It is known to possess an impressive biological activity profile including antibiotic and HIV inhibitory activities, as well as cytotoxic and mammalian DNA binding properties (34). Equisetin exists as two epimers - equisetin (EQ) and epiequisetin (epi-EQ) (47). EQ and its C-5′ epimer epi-EQ have been reported to be majorly produced by cultures of *F. equiseti* (34, 46-48). However, it has also been isolated from other *Fusarium* species such as *F. pallidoroseum* (47) and *F. heterosporum* (31).

EQ demonstrates antibiotic and cytotoxic activities (48-50). EQ inhibits mitochondrial ATPase activity (31,51). It inhibits HIV-1 integrase (31, 34, 52, 53). EQ has been reported to show strong antibiotic activity against some Gram-positive bacteria and mycobacterium (46). Epi-EQ, the phytotoxic isomer of EQ, is also reported to inhibit HIV-1 integrase (53). Wheeler *et al.* (47) reported the phytotoxicity of the EQ and epi-EQ to certain plants during seed germination or seedling growth.

Compounds with anticancer properties also isolated from *F. equiseti* include Diacetoxyscirpenol (54, 55) and Fusarochromanone and its derivatives (56, 57). Other compounds from *F. equiseti* are 4-acetylnivalenol, nivalenol, scirpentriol zearalenone, beauvericin, fusarochromanone, equisetine and butenolide (58, 59).

Epicoccum sorghinum (Didymellaceae), formerly known as *Phoma sorghina* (60), is a facultative plant pathogen considered to be majorly associated with the sorghum grain-mold disease complex (61). The fungus is also the cause of leaf spot disease of plants such as *Eichhornia crassipes* (62), *Colocasia esculenta* (63), *Nicotiana tabacum* (64), *Oxalis debilis* (65), and *Phytolacca Americana* (66). *E. sorghinum* is known to produce the mycotoxin tenuazonic acid (TA) (61, 66-68). Several other phytotoxins such as epoxydon, desoxyepoxydon, phyllostine, diphenyl ether, and 6-methyl salicyalate ether have been expressed by the fungus (66). In contrast to its pathogenic activity on sorghum grains, *E. sorghinum* is reported to be the most prevalent endophytic fungal species associated with the leaves of sorghum field plants (69, 70). It has also been found as endophytes of *Annona senegalensis* (71), *Rhodomyrtus tomentosa* (61), and *Tithonia diversifolia* (66).

TA a tetramic acid derivative, is a mycotoxin and phytotoxin majorly produced by *Alternaria alternata* (*A. tenuis*), as well as by other phytopathogenic *Alternaria* species (36, 68, 72, 73). The compound is also expressed by other species of fungi such as *E. sorghinum* and *Pyricularia oryzae* (36, 68). TA inhibits protein biosynthesis, and it's the most toxic of the *Alternaria* toxins (68, 74). TA is a non-specific phytotoxin as it exhibits significant phytotoxic effects on both monocotyledonous and dicotyledonous plants (75-77). TA is also toxic to mice, chick embryos and chickens (36). The compound has been implicated as a possible cause or contributing factor to 'Onyalai', a human

hematological disorder occurring in Africa after consumption of sorghum (78). TA has shown antiviral, antitumor, antibiotic, and phytotoxic activities (35, 36, 75, 79). The compound possesses broad spectrum and quick killing properties, and as such can be employed as a bioherbicide (72, 77).

Apart from expressing these biologically important compounds, these two toxigenic endophytic fungi isolated from *C. papaya* are reported to have industrial applications. *F. equiseti* and *E. sorghinum* have been shown to have potentials for use as biocontrol agents of weeds and plant pathogens (62,80-84). *F. equiseti* is also employed as a plant growth promoting fungus (82,83). Also, an *F. equiseti*-dreived protease showed excellent stain removal property and was compatible with several commercial laundry detergent formulations (85).

Conclusion

F. equiseti and *E. sorghinum* isolated from *C. papaya* produced toxic compounds known to have beneficial potentials for pharmaceutical, agricultural or industrial purposes.

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Conflict of interest statement

The authors declare no conflict of interest.

References

1. Saeed F, Arshad MU, Pasha I, Naz R, Batool R, Khan AA, Nasir MA, Shafique B. Nutritional and Phyto-Therapeutic Potential of Papaya (*Carica papaya* Linn.): An Overview. International Journal of Food Properties 2014; 7:1637–1653.
2. Gurunga S, Škalko-Basnet N. Wound healing properties of *Carica papaya* latex: *In vivo* evaluation in mice burn model. Journal of Ethnopharmacology 2009; 121:338–341.
3. Mikhalchik EV, Ivanova AV, Anurov MV, Titkova SM, Penkov LY, Kharaeva ZF, Korkina LG. Wound-healing effect of papaya-based preparation in experimental thermal trauma. Bulletin of Experimental Biology and Medicine 2004; 137:560–562.
4. Owoyele BV, Adebukola OM, Funmilayo AA, Soladoye AO. Anti-inflammatory activities of ethanolic extract of *Carica papaya* leaves. Inflammopharmacol. 2008; 16:168-173.
5. Amazu LU, Azikiwe CCA, Njoku CJ, Osuala FN, Nwosu PJC, Ajugwo AO, Enye JC. Anti-inflammatory activity of the methanolic extract of the seeds of *Carica papaya* in experimental animals. Asi. Pac.J Trop. Med. 2010:884-886.
6. Lohiya NK, Manivannan B, Mishra PK, Pathak N, Sriram S, Bhande SS, Panneerdoss S. Chloroform extract of *Carica papaya* seeds induces long term reversible azoospermia in langur monkey. Asian J Androl. 2002; 4(1):17-26.
7. Poharkar RD, Saraswat RK, Kotkar S. Survey of plants having anti-fertility activity from western Ghat area of Maharashtra state. J Herb Med Toxicol. 2010; 4(2):71-75.
8. Stepek G, Behnke JM, Buttle DJ, Duce IR. Natural plant cysteine proteinases as anthelmintics. Trends in Parasitology. 2004; 20(7):322-327.
9. Desser L, Rehberger A, Kokron E, Paukovits W. Cytokine synthesis in human peripheral blood mononuclear cells after oral administration of polyenzyme preparation. J Oncology. 1993; 50:403-407.
10. Fauziya S, Krishnamurthy R. Papaya (*Carica papaya*): Source material for anticancer. CIBTech J Pharm *Sci.* 2013; 2(1):25-34.
11. Doughari J, Elmahmood AM, Manzara S. Studies on the antibacterial activity of root extracts of *Carica papaya* L. Afr. J Microbiol. Res. 2007:37-41.
12. Emeruwa AC. Antibacterial substance from *Carica papaya* fruit extract. J Nat Prod. 1982; 45(2):123-127.
13. Leite AA, Nardi RM, Nicoli JR, Chartone SE, Nascimento AM. *Carica papaya* seed macerate as inhibitor of conjugative R plasmid transfer from *Salmonella typhimurium* to *E. coli, in vitro* and in the digestive tract of genobiotic mice. J Gen Appl Microbiol. 2005; 51(1):21-26.
14. Giordiani R, Siepaio M, Moulin TJ, Regli P. Antifungal action of *Carica papaya* latex, isolation of fungal cell wall hydrolyzing enzymes, Mycoses. 1991; 34(11-12):467-477.
15. N'guessan K, Tiébré M, Aké-Assi E, Zirihi GN. Ethnobotanical study of plants used to treat arterial hypertension, in traditional medicine by Abbey and Krobou populations of Aboville (Côte-d'Ivoire). European. J Scient Res. 2009; 35(1):85-98.
16. Mojica-Henshaw MP, Francisco AD, Deguzman F, Tingo T. Possible Immunomodulatory action of *Carica papaya* seed extract. Clin Hemorheol Microcirc. 2003; 29(3-4):219-229.
17. Titanji VP, Zofou D, Ngemenya MN. The Antimalarial Potential of Medicinal Plants Used for the Treatment of Malaria in Cameroonian Folk Medicine. Afr. J. Tradit. Complement. Altern. Med. 2008; 5(3):302–321.
18. Bungorn S, Varima W, Pisamai L, Jamsai S, Dusit J. Diuretic effects of selected Thai indigenous medicinal plants in rat. J Ethnopharmacol. 2001; 75(2-3):185-190.
19. Wright CI, Van-Buren L, Kroner CI, Koning MMG. Herbal medicines as diuretics: A review of the scientific evidence. J Ethnopharmacol. 2007; 114:1-31.
20. Mailafia S, Okoh GR, Olabode HOK, Osanupin R. Isolation and identification of fungi associated with spoilt fruits vended in Gwagwalada market, Abuja, Nigeria, Veterinary World 2017, *10(4),* 393-397.
21. Echerenwa MC, Umechuruba CI. Post-harvest fungal diseases of pawpaw (*Carica papaya* L.) fruits and seeds in Nigeria. Global Journal of Pure and Applied Sciences 2004; 10(1):69-73.
22. Oyeyipo OO, Iwuji CA, Owhoeli O. Public Health Implication of Mycotoxin Contaminated Pawpaw (*Carica papaya* L) on Sale in Nigerian Markets. International Journal of Health Research 2012; 5(1):23-27.
23. Gupta AK, Pathak VN. Survey of fruit market for papaya fruit rot by fungi pathogens. Indian J Mycol 1986; *16*:152-254.
24. Oniha M, Egwari L. Fruit, Leaf and Stem Diseases of *Carica papaya* L. Journal of International Scientific Publications 2015; *3*:398-407.
25. Krishnan P, Bhat R, Kush A, Ravikumar P. Isolation and functional characterization of bacterial endophytes from *Carica papaya* fruits. Journal of Applied Microbiology 2012; 113:308-317.
26. Mello NRTD, Cavalcanti MS, Ferreira MRV, Oliveira WCR, Ribeiro IATA, Santos IP, Silva APS. Enzymatic Activity of Endophytic Fungi Isolated from Papaya (*Carica papaya* L.). 4th International Symposium in Biochemistry of Macromolecules and Biotechnology. XI Northeast Regional Meeting of SBBq, Recife, PE, Brazil; December 5 to 7, 2012.
27. Okezie UM, Eze PM, Ajaghaku DL, Okoye FBC, Esimone CO. Isolation and screening of secondary metabolites from endophytic fungi of *Vernonia amygdalina* and *Carica papaya* for their cytotoxic activity. Planta Med. 2015; 81:PM_177.
28. Wang H, Eze PM, Höfert S, Janiak C, Hartmann R, Okoye FBC,

Esimone CO, Orfali RS, Dai H, Liu Z, Proksch P. Substituted L-tryptophan-L-phenyllactic acid conjugates produced by an endophytic fungus *Aspergillus aculeatus* using an OSMAC approach. RSC Adv. 2018; 8:7863–7872.
29. Eze PM, Ojimba NK, Abonyi DO, Chukwunwejim CR, Abba CC, Okoye FBC, Esimone CO. Antimicrobial Activity of Metabolites of an Endophytic Fungus Isolated from the Leaves of *Citrus jambhiri* (Rutaceae). Trop. J. Nat. Prod. Res. 2018; 2(3):145-149.
30. Kjer J, Debbab A, Aly AH, Proksch P. Methods for isolation of marine-derived endophytic fungi and their bioactive secondary products. Nat. Protoc. 2010; *5*:479–490.
31. Singh SB, Zink DL, Goetz MA, Dombrowskia AW, Polishooka JD, Hazuda DJ. Equisetin and a novel opposite stereochemical homolog phomasetin, two fungal metabolites as inhibitors of HIV-1 integrase. Tetrahedron Lett. 1998; 39:2243-2246.
32. Whitt J, Shipley SM, Newman DJ, Zuck KM. Tetramic Acid Analogues Produced by Coculture of *Saccharopolyspora erythraea* with *Fusarium pallidoroseum*. J. Nat. Prod. 2014; 77:173−177.
33. Phillips NJ, Goodwin JT, Fraiman A, Cole RJ, Lynn DG. Characterization of the Fusarium toxin equisetin: the use phenylboronates in structure assignment. J. Am. Chem. Soc. 1989; 111(21):8223–8231.
34. Burke LT, Dixon DJ, Ley SV, Rodrıguez F. Total synthesis of the *Fusarium* toxin equisetin. Org. Biomol. Chem. 2005; 3:274–280.
35. Davis ND, Diener UL, Morgan-Jones G. Tenuazonic Acid Production by Alternaria alternata and Alternaria tenuissima Isolated from Cotton. Applied and Environmental Microbiology 1977; 34(2):155-157.
36. Logrieco A, Bottalico A, Mulé G, Moretti A, Perrone G. Epidemiology of toxigenic fungi and their associated mycotoxins for some Mediterranean crops. European Journal of Plant Pathology 2003; 109:645–667.
37. Katoch M, Salgotra A, Singh G. Endophytic fungi found in association with *Bacopa monnieri* as potential producers of industrial enzymes and antimicrobial bioactive compounds. Brazilian Archives of Biology and Technology. 2014; 57(5):714-722.
38. Devaraju R, Sreedharamurthy S. Endophytic Mycoflora of *Mirabilis jalapa* L. and studies on Antimicrobial activity of its endophytic *Fusarium* sp. Asian J Exp Biol Sci. 2011; 2:75-79.
39. Waskiewicz A, Golinski P, Karolewski Z, Irzykowska L, Bocianowski J, Kostecki M, Weber Z. Formation of fumonisins and other secondary metabolites by *Fusarium oxysporum* and *F. proliferatum*: a comparative study. Food Addit. Contam. Part A, Chem. Anal. Control Expo Risk Assess. 2010; 27(5):608-15.
40. Tatum JH, Baker RA, Berry RE. Metabolites of *Fusarium solani.* Phytochemistry. 1989; 28(1):283-284.
41. Savard ME, Miller JD, Ciotola M, Watson AK. Secondary Metabolites Produced by a Strain of *Fusarium oxysporum* used for Striga Control in West Africa. Biocontrol Science and Technology. 1997: 7(1):61-64.
42. Hernandes L, Marangon AV, Salci T, Svidzinski TIE. Toxic thermoresistant metabolites of *Fusarium oxysporum* are capable of inducing histopathological alterations in Wistar rats. *The Journal of Venomous Animals and Toxins including* Tropical Diseases. 2012; 18(2):144-149.
43. Goswami RS, Dong Y, Punja ZK. Host range and mycotoxin production by *Fusarium equiseti* isolates originating from ginseng fields. Canadian Journal of Plant Pathology. 2008; 30(1):155-160.
44. Marín P, Moretti A, Ritieni A, Jurado M, Vázquez C, González-Jaén MT. Phylogenetic analyses and toxigenic profiles of *Fusarium equiseti* and *Fusarium acuminatum* isolated from cereals from Southern Europe. Food Microbiol. 2012; 31(2): 229-37.
45. Langseth W, Bernhoft A, Rundberget T, Kosiak B, Gareis M. Mycotoxin production and cytotoxicity of Fusarium strains isolated from Norwegian cereals. Mycopathologia 1998; 144:103-113.
46. Burmeister HR, Bennett GA, Vesonder RF, Hesseltine CW. Antibiotic production by *Fusarium equiseti* NRRL 5537. Antimicrobial Agents and Chemotherapy. 1974; 5:634-639.
47. Wheeler MH, Stipanovic RD, Puckhaber LS. Phytotoxicity of equisetin and epi-equisetin isolated from *Fusarium equiseti* and *F. pallidoroseum*. Mycological Research 1999; 103(8):967–973.
48. Vesonder RF, Tjarks LW, Rohwedder WK, Burmeister HR, Laugal JA. Equisetin, an antibiotic from *Fusarium equiseti* NRRL 5537, identified as a derivative of N-methyl-2,4-pyrollidone. J Antibiot (Tokyo) 1979; 32(7):759-61.
49. Desjardins AE, Proctor RH. Molecular biology of Fusarium mycotoxins. Int J Food Microbiol. 2007; 119:47-50.
50. Patham B, Duffy J, Lane A, Davis RC, Wipf P, Fewell SW, Brodsky JL, Mensa-Wilmot K. Post-translational import of protein into the endoplasmic reticulum of a trypanosome: An *in vitro* system for discovery of anti-trypanosomal chemical entities. Biochem J. 2009; 419(2):507-517.
51. König T, Kapus A, Sarkadi B. Effects of equisetin on rat liver mitochondria: evidence for inhibition of substrate anion carriers of the inner membrane. J Bioenerg Biomembr. 1993: 25(5):537-45.
52. Tziveleka LA, Vagias C, Roussis V. Natural products with anti-HIV activity from marine organisms. Curr. Top. Med. Chem. 2003: 3(13):1512-35.
53. Singh, S.B. Discovery and development of natural product inhibitors of HIV-1 integrase. In HIV-1 Integrase: Mechanism and Inhibitor Design. Neamat N (Ed). John Wiley & Sons, 2011.
54. Brian PW, Dawkins AW, Grove JF, Hemming HG, Lowe D, Norris GLF. Phytotoxic Compounds produced by *Fusarium equiseti.* Journal of Experimental Botany 1961; 13(34):1-12.
55. Dosik GM, Barlogie B, Johnston DA, Murphy WK, Drewinko B. Lethal and cytokinetic effects of anguidine on a human colon cancer cell line. Cancer Res. 1978; 38(10):3304-9.
56. Xie W, Mirocha CJ, Wen Y. Formyl Fusarochromanone and Diacetyl Fusarochromanone, Two New Metabolites of *Fusarium equiseti*. Journal of Natural Products. 1991; 54(4):1165-1167.
57. Mahdavian E, Palyok P, Adelmund S, Williams-Hart T, Furmanski BD, Kim Y, Gu Y, Barzegar M, Wu Y, Bhinge KN, Kolluru GK, Quick Q, Liu Y, Kevil CG, Salvatore BA, Huang S, Clifford JL. Biological activities of fusarochromanone: a potent anti-cancer agent. BMC Research Notes 2014; 7:601.
58. Thrane U. *Fusarium* species and their specific profiles of secondary metabolites. In: Chelkowski J, editor. *Fusarium*—mycotoxins, taxonomy and pathogenicity. Amsterdam: Elsevier 1989:199–226.
59. Pillai TG, Nair B, Swamy GEM. Isolation of Host Specific Endophytic Fungus, *Fusarium equiseti*, from Nothopegia Bedomei, Wayanadica Occurring in the Southern Parts of India. J. Plant Pathol. Microb. 2015; 6:308.
60. Aveskamp MM, de Gruyter J, Woudenberg JHC, Verkley GJM, Crous PW. Highlights of the *Didymellaceae*: a polyphasic approach to characterize *Phoma* and related pleosporalean genera. Stud. Mycol. 2010; 65:1– 60.
61. Li C, Sarotti AM, Yang B, Turkson J, Cao S. A New N-methoxypyridone from the Co-Cultivation of Hawaiian Endophytic Fungi Camporesia sambuci FT1061 and Epicoccum sorghinum FT1062. Molecules 2017, *22(1166),* 1-8.
62. Ray P, Sushilkumar, Pandey AK. Survey and selection of potential pathogens for biological control of water hyacinth. Indian Journal of Weed Science 2008; 40(1&2):75-78.
63. Liu PQ, Wei MY, Zhu L, Wang RB, Li BJ, Weng QY, Chen QH. First Report of Leaf Spot on Taro Caused by *Epicoccum sorghinum* in China. Plant Disease 2018; 102(3):682
64. Yuan CG, Liao T, Tan HW, Li QQ, Lin W. First Report of Leaf Spot Caused by *Phoma sorghina* on Tobacco in China. Plant Disease 2016; 100(8):1790.

65. Chen XL, Wang YH, Luo T. First Report of Leaf Spot Caused by *Phoma sorghina* on *Oxalis debilis* in China. Plant Disease 2017; 101(6):1047
66. Rai M, Deshmukh P, Gade A, Ingle A, Kövics GJ, Irinyi L. *Phoma Saccardo*: Distribution, secondary metabolite production and biotechnological applications. Critical Reviews in Microbiology 2009; 35(3):182–196.
67. Oliveira RC, Davenport KW, Hovde B, Silva D, Chain PSG, Correa B, Rodrigues DF. Draft Genome Sequence of Sorghum Grain Mold Fungus *Epicoccum sorghinum*, a Producer of Tenuazonic Acid. Genome Announc. 2017; 5(4):e01495-16.
68. Yun C, Motoyama T, Osada H. Biosynthesis of the mycotoxin tenuazonic acid by a fungal NRPS–PKS hybrid enzyme. Nature Communications 2015, 6:8758.
69. Zida EP, Néya JB, Soalla WR, Jensen SM, Stokholm MS, Andresen M, Kabir MH, Sérémé P, Lund OS. Effect of sorghum seed treatment in Burkina Faso varies with baseline crop performance and geographical location. Afr. Crop Sci. J. *2016*; 24:109–125.
70. Stokholm MS, Wulff EG, Zida EP, Thio IG, Néya JB, Soalla RW, Głazowska SE, Andresen M, Topbjerg HB, Boelt B, Lund OS. DNA barcoding and isolation of vertically transmitted ascomycetes in sorghum from Burkina Faso*: Epicoccum sorghinum* is dominant in seedlings and appears as a common root pathogen. Microbiological Research 2016; 191:38–50.
71. Sanodiya BS, Thakur GS, Baghel RK, Pandey AK, Prasad GBKS, Bisen PS. Isolation and characterization of tenuazonic acid produced by *Alternaria alternata*, a potential bioherbicidal agent for control of *Lantana camara.* Journal of Plant Protection Research 2010; 50(2):133-139.
72. Sibanda EP, Mabandla M, Mduluza T. Antioxidant activity of fungal endophytes isolated from *Kigelia africana, Annona senegalensis* and *Vitex payos*. Microbiology: Current Research 2017; 1(2):61.
73. Rosett T, Sankhala RH, Stickings CE, Taylor MEU, Thomas R. Biochemistry of microorganisms. CIII. Metabolites of *Alternaria tenuis auct*: Culture filtrate products. Biochem. *J.* 1957; 67:390–400.
74. Shigeura HT, Gordon CN. The biological activity of tenuazonic acid. Biochemistry 1963; 2:1132–1137.
75. Janardhanan KK, Husain A. Phytotoxic activity of tenuazonic acid isolated from *Alternaria alternata* (Fr.) Keissler causing leaf blight of *Datura innoxia* Mill. and its effect on host metabolism. J. Phytopathol. 1984; 111:305–311.
76. Umetsu N, Muramatsu T, Honda H, Tamari K. Studies of the Effect of Tenuazonic Acid on Plant Cells and Seedlings. Agr. Biol. Chem. 1974; 38(4):791 – 799.
77. Meena, M.; Swapnil, P.; Upadhyay, R.S. Isolation, characterization and toxicological potential of *Alternaria* mycotoxins (TeA, AOH and AME) in different *Alternaria* species from various regions of India. Scientific Reports 2017; 7:8777.
78. Steyn, P.S., Rabie, C.J. Characterization of magnesium and calcium tenuazonate from *Phoma sorghina*. Phytochemistry. 1976; 15:1977-1979.
79. Suzuki S, Sano F, Yuki H. Studies on antiviral agents. IV. Biological Activity of Tenuazonic Acid derivatives. Chem. Pharm. Bull. 1967; 15(8):11220-1122.
80. Motlagh MRS. *Fusarium equiseti* (Corda) Saccardo as biological control agent of barnyard grass (*Echinochloacrus galli* L.) in rice fields. Food, Agriculture and Environment 2011; 9(1):310-313.
81. Horinouchi H, Muslim A, Suzuki T, Hyakumachi M. *Fusarium equiseti* GF191 as an effective biocontrol agent against Fusarium crown and root rot of tomato in rock wool systems. Crop Protection. 2007; 26:1514-1523.
82. Horinouchi H, Katsuyama N, Taguchi Y, Hyakumachi M. Control of Fusarium crown and root rot of tomato in a soil system by combination of a plant growth-promoting fungus, *Fusarium equiseti,* and biodegradable pots. Crop Protection 2008; 27:859-864.
83. Horinouchi H, Muslim A, Hyakumachi M. Biocontrol of Fusarium Wilt of Spinach by the Plant Growth Promoting Fungus *Fusarium equiseti* GF183. Journal of Plant Pathology. 2010; 92(1):249-254.
84. Abbasher AA, Hess DE, Sauerborn J. Fungal pathogens for biological control of *Striga hermonthica* on sorghum and pearl millet in West Africa. African Crop Science Journal 1998; 6(2):179-188.
85. Juntunen K, Mäkinen S, Isoniemi S, Valtakari L, Pelzer A, Jänis J, Paloheimo M. A New Subtilase-Like Protease Deriving from *Fusarium equiseti* with High Potential for Industrial Applications. Appl Biochem Biotechnol. 2015; 177(2):407–430.