FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 5 No. 3, September, 2021, pp 281- 285
DOI: https://doi.org/10.33003/fjs-2021-0503-687

PHYTOCHEMICAL, ANTIOXIDANT AND ANTIMICROBIAL STUDIES OF THE CRUDE EXTRACT OF Curvulariasp. ISOLATED FROM THE LEAVES OF THE MEDICINAL PLANT Piliostigma thonningii (Fabaceae)

[1,2]Ogbiko, C., [3]Eboka, C.J., [4]Okezie, U.M. and [2]Okoye, F.B.C.

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Madonna University Elele, Rivers State Nigeria.
[2]Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, NnamdiAzikiwe University Awka, Anambra State Nigeria.
[3]Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, University of Benin Benin City, Edo State Nigeria.
[4]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, NnamdiAzikiwe University Awka, Anambra State Nigeria.
*Corresponding author's email: cyrilogbiko@gmail.com +234-808-0398-933

ABSTRACT
Recently, there is biotechnological interest in secondary metabolites of endophytes since they have been shown to be capable of producing substances of pharmacological importance. Piliostigmathonningii is a tree whose parts are widely employed in Nigeria for diverse medicinal applications. The aim of this study is to investigate the phytochemical composition, the antiradical and in vitro antimicrobial potentials of the crude ethyl acetate extract of Curvularia sp isolated from the leaves of the plant. The endophytic fungus was isolated, purified, and identified using conventional methods. While standard procedures was employed in the determination of the phytochemical constituents, quantitative estimation of the total phenol and flavonoid were done by spectrophotometric method using gallic acid and quercetin as standards respectively. While the antioxidant activity was determined by DPPH scavenging assay, the agar well diffusion method was employed to screen for its antimicrobial capabilities. Results showed the presence ofimportant phytoconstituents notably alkaloid, flavonoid, phenolics, tannin, saponins among others. While the antioxidant assay showed the total phenolic and flavonoid contents to be 212.55 ± 1.2 mgGAE/g extract and 143.16 ± 0.8 mgQE/g extract respectively, a 74.45 ± 0.11% free radical scavenging activity was observed compared to ascorbic acid standard that exhibited a 87.92 ± 0.17% inhibition. At the highest investigated concentration of 1 mg/mL, the extract produced antibacterial activity only against Escherichia coliandPseudomonas aeruginosa. The results showed that the extract exhibits marked antioxidant and selected antibacterial effects thus justifying the assertion that endophytic bioactive compounds have promising applications in the field of medicine.

Keywords: Antioxidant, Antimicrobial, Curvulariasp, Phytochemical, Secondary Metabolites

INTRODUCTION

Free radicals are chemically aggressive molecules that have been implicated for damages to different macromolecules in the body such as DNA, proteins and lipids (Harman, 1981). They contribute to numerous disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS (Kumpulainen and Salonen, 1999; Cook and Samman, 1996). Cells are equipped with natural antioxidant systems like α-tocopherol, β-carotene, ascorbic acid as well as synthetic antioxidants like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tert-butylhydroquinone (TBHQ) whose mechanisms are primarily to scavenge these free radicals to maintain the redox homeostasis of cells (Farrukh et al., 2006). The increase in global chemotherapeutic failure could be attributed to the antimicrobial toxicity and undesirable side effects of the present antibiotics employed to curb the present and emerging infections (Ogbiko et al., 2020). As a result of the unbearable side effects, carcinogenicity, high cost and inaccessibility, there is an inspired search for more effective antioxidant from natural source, especially from medicinal plants known to be biodegradable, less toxic, affordable and readily accessible (Deepa and Renuka, 2014; Ezeja et al., 2015). Natural products have long been recognized as specialized product structures possessing high chemical diversity, biochemical specificity and other molecular properties that make them favorable as lead structures for drug discovery (Okoye and Osadebe, 2010).

Piliostigmathonningii, Schum (Milne-Redhead) of the family Fabaceae commonly known as monkey's bread or camel's foot tree and locally known as ''kalgo'' (Hausa), ''kalur'' (Kanuri) and ''inpilataki'' (Higgi) have long been used in the management and treatment of inflammation, bilharzia, eye diseases, catarrh malaria fever among others (Kwaji et al., 2010). Endophytic fungal populations from Nigerian medicinal plants have been shown to possess enormous potentials as sources of biologically active compounds of pharmaceutical and industrial importance (Chika etal., 2018). It is estimated that there might be as many as one million different endophytic fungal species, however, only a handful of them have been described, which implies that investigating the metabo¬lites of endophytes for pharmacological activities will enhance the chance of finding novel bioactive compounds (Okoye et al., 2015). There is the need to explore medicinal plants endophytic population for biologically important molecules, hence our study seeks to phytochemically profile and ascertain the in vitro antioxidant and antimicrobial potential of the secondary metabolites of an endophytic fungus isolated from the leaves of P. thonningii growing in Sokoto State, North-West Nigeria.

MATERIALS AND METHODS

Plant material collection, authentication and preparation

Fresh and healthy leaves of P. thonningiiwere collected from Tambuwal local government area of Sokoto State in April, 2020. Taxonomic identification was done by Mallam Abdulaziz Salihu of Botany Unit, Department of Biological Sciences, Faculty of Science, Usmanu Danfodiyo University Sokoto. Herbarium specimen was prepared, deposited and voucher number UDUH/ANS/0137 issued. The fresh leaves were prepared and transported to the Department of Pharmaceutical Microbiology and Biotechnology, Nnamdi Azikiwe University Awka, Anambra State for the endophytic fungus isolation and purification.

Isolation of endophytic fungus

Isolation of the endophytic fungus from the plant leaves, solid-state fermentation of the fungus and extraction of the fungal metabolites were carried out using methods previously described by Akpotu et al., 2017a and Akpotuet al., 2017b. Selected healthy plant leaves were washed thoroughly in running tap water and processed as follows: the leaves were cut into 1 cm fragments and surface-sterilized by immersion in 2% sodium hypochlorite solution for 2 min, 70% ethanol for nearly 2 min, before a final rinse in sterile water for 5 min. The leaf fragments were placed on Petri plates containing malt extract agar (MEA) supplemented with chloramphenicol. The plates were then incubated at 28oC temperature and fungal growth from the leaf fragments was monitored. Hyphal tips from distinct colonies emerging from leaf segments were sub-cultured onto fresh MEA plates to obtain pure colonies.

Fermentation and extraction of endophyte secondary metabolites

Solid state fermentation of the endophytic fungus was carried out in 1L Erlenmeyer flask containing autoclaved rice medium (100 g of rice and 200 mL of distilled water). The flask was inoculated with 3 mm diameter agar blocks containing the fungi and incubated at 28°C for 21 days. At the completion of fermentation, the secondary metabolites were extracted in ethyl acetate and then concentrated under vacuum at 40oC using a rotary evaporator.

Phytochemical screening

The phytochemical screening wasconducted on the crude secondary metabolite in accordance to standardprocedures (Khandewal, 2000).

Determination of total phenolic content

The total phenolic content in the extract was determined according to the FolinCiocalteu procedure outlined by Kim et al., 2003 with slight modifications. The extract solution (0.5 mL) with a concentration of 1000 µg/mL was added to 4.5 mL of deionized distilled water and 0.5 ml of FolinCiocalteu's reagent previously diluted with deionized water (1:10 v/v). The solution maintained at room temperature for 5 minutes was followed by the addition of 5 ml of 7% sodium carbonate and 2 mL of deionized distilled water and mixed thoroughly. The samples were incubated for 90 minutes at 23°C.with the absorbance measured with a UV-VIS spectrophotometer at 750 nm. The total phenolic content was expressed as milligrams of gallic acid equivalents (GAE) per gram of extract. Gallic acid was used as the positive control. The standard curve was prepared by gallic acid in five different concentrations (50, 100, 150, 250 and 500 mg/L).

Determination of total flavonoid content

The total flavonoid content in the plant extracts was determined by the Aluminum Chloride method as outlined by Ebrahimzadehet al., 2008. 0.5 ml of the extract (5 g/L) was mixed with 1.5 ml of methanol and then 0.1 ml of 10 % aluminum chloride was added followed by 0.1 ml of potassium acetate and 2.8 ml of distilled water. The mixture was incubated at room temperature for 30 min. The absorbance was measured by a spectrophotometer at 415 nm. The results were expressed as milligrams quercetin equivalents (QE) per gram of extract (mg QE/g dry extract). Quercetin was used as positive control and the standard curve was prepared by quercetin in different concentrations (12.5, 25, 50, 80, and 100 mg/L).

Screening of the crude extract for free radical scavenging activity

The ability of plant extracts to scavenge 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radicals was estimated as previously described by Jain et al., 2008. The extracts (3 mL) with five different concentrations (12.50, 25.00, 50.00, 100.00, and 200.00µg/ml) were mixed with 1 ml of a 0.1 mM methanol solution of DPPH. Each of the mixtures was shaken vigorously, and then incubated in the dark at room temperature for 30 min. The UV absorbance was measured at 517 nm using a UV-VIS spectrophotometer. 2 mL of MeOH solvent mixed with 2 mL of DPPH solution served as blank (negative control) while ascorbic acid at the same concentrations was used as the positive control. The DPPH free radical scavenging effect of the samples was calculated using the following formula:

DPPH radical inhibition (%) = [1 − (A test/ A control)] × 100

Where $A_{control}$ is the absorbance of the control and A_{test} is the absorbance of the tested extracts. The antioxidant activity of the sample was also expressed in terms of IC50 value, which represents the concentration of the sample required to inhibit 50% formation of DPPH radical.

Antimicrobial assay

Antibacterial and antifungal screening of 1 mg/mL concentration of the crude extract of the fungus was prepared using standard procedures and agar well diffusion method previously described by Akpotuet al., 2017 (a) and (b)b. was employed to ascertain its antimicrobial activity. The extract was challenged with confirmed laboratory strains of Staphylococcus aureus; Bacillus subtilis; E. coli; Pseudomonas aeruginosa; Salmonella species; Klebsiellapneumoniae, Candida albicans, and Aspergillus nigerprocured from the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences NnamdiAzikiwe University Awka Anambra State. The inhibition zone diameters (IZDs) were measured and recorded. The procedure was conducted in triplicate, and the mean IZDs calculated.

Statistical analysis

In the antioxidant assays, UV absorbance was measured in triplicates with the average calculated and recorded. They are expressed as mean ± standard error of mean (SEM) with statistical comparisons made using the student t-test. Confidence limit was set at $p < 0.05$.

RESULTS AND DISCUSSION

Phytochemical screening

Preliminary phytochemical analysis showed the presence of major classes of secondary metabolites as presented in Table 1.

Table 1: Phytochemical screening of the crude EtOAc extract of Curvulariasp isolated from the leaves of P. thonningii

Phytochemical	Inference
Alkaloids	+
Saponins	+
Flavonoids	+
Phenolics	+
Tannins	+
Carbohydrate	+
Reducing sugars	+
Terpenoid	-
Cardiac glycosides	-

Key: (+) = present; (-) = absence of phytoconstituents

Preliminary phytochemical screening of medicinal plants to reveal their phytoconstituents is a valuable step in the detection of the bioactive principles often explored in drug discovery and development (Jain et al., 2008). The crude ethyl acetate extract of the endophyte isolated from P. thonningii revealed the presence of various phytochemicals of pharmacological relevance.

DPPH and polyphenolic content

Table 2 and Figure 1 shows the results of the polyphenolic contents (determined from the gallic acid (R2 = 0.9773) and quercetin (R2 = 0.9812) calibration curves) and DPPH antioxidant assay of the fungal extract respectively.

Table 2: Total phenolics and flavonoids content of the crude EtOAc extract of Curvulariaspendophyte isolated from P. thonningiileaves

Total phenolic content*	212.55 ± 1.2
Total flavonoid content**	143.16 ± 0.8

*mg gallic acid equivalent (mgGAE)/g extract; **mg Quercetin equivalent (mgQE/g extract)

Values are means of three biological replicates.

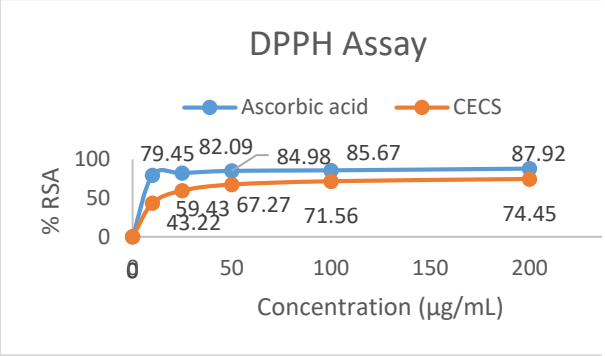

Figure 1: DPPH radical scavenging activity of crude ethyl acetate extract of the endophytic fungal and the synthetic antioxidant (ascorbic acid) at different concentrations. **Each point is a mean from triplicate measurement. CECS = crude extract of *Curvulariasp***

Natural antioxidants have been established to be pivotal in the management of disorders associated with oxidative stress (Yadav et al., 2014). The result of the DPPH assay of the CECS showed an increase in the radical scavenging ability in a dose dependent manner although lower than the synthetic antioxidant. This could be strongly linked to the phenolic and flavonoid contents (Table 2). Polyphenolic compounds like phenols, flavonoids, phenolic acids, stillbenes, tannins, lignins among others are known to scavenge radicals such as singlet oxygen, superoxide, and hydroxyl because of their phenolic hydroxyl groupsthat allow them to act as antioxidants (Gandía-Herrero et al., 2009; Vijay and Vimukta, 2014). This agreed to the report of several researchers who stated that plants with high contents of phenolics and flavonoids had high antioxidant activities (Maisuthisakul et al., 2007; Maisuthisakul et al., 2008; Zhang and Lin, 2008; Zhang et al., 2015).

Antimicrobial Activity

The results presented in Table 3 show the in vitro susceptibility of the microbial isolates of the crude extract of the endophytic fungus in comparison to ciprofloxacin (5 µg/mL) and miconazole (50 µg/mL) standards respectively. The ethyl acetate crude extract of the endophytic fungus at 1.00 mg/mL showed activity only when tested against Escherichia coli and Pseudomonas aeruginosa with no inhibition noticed for all other microbes. There were significant differences between the IZDs of the standard antibiotics and the extract for the sensitive microbes.

Table 3: Result of antimicrobial activities (inhibition zone diameter) of the crude extract of Curvulariaspp isolated from the leaves of P. thonningii

Drugs	Microorganisms/ IZDs (mm) at 1.00 mg/mL							
	SA	BS	EC	PA	SS	KP	CA	AN
CECS	0	0	2±0.7[a]	2.5±0.7[a]	0	0	0	0
CIP 5µg/mL	15±0.7	17±0	18±0.[7b]	11±0[b]	15±0.7	15±0.7	ND	ND
MICO 50 µg/mL	ND	ND	ND	ND	ND	ND	14±0	11±0.4
DMSO (NC)	0	0	0	0	0	0	0	0

Key: SA (Staphylococcus aureus); BS (Bacillus subtilis); EC (Escherichia coli); PA (Pseudomonas aeruginosa); SS (Salmonella species); KP (Klebsiellapneumonia); CA (Candida albicans); AN (Aspergillus niger); CECS = Crude Extract of Curvulariaspp; CIP = Ciprofloxacin; MICO = Miconazole; DMSO = Dimethyl sulphoxide; NC = Negative Control; ND = Not Determined. Values are presented as mean ± standard error of mean of three determinations; Values with different superscript in the same column are significantly different at $p < 0.05$.

The antibacterial activity test as depicted by the inhibition zone diameter was seen to be concentration-dependent. The reported antimicrobial activity for the extract and fractions may be due to the presence of the detected phytochemicals (Table 1) especially phenols, flavonoids, tannins andsaponins that were all detected in the extract have been shown to have antibacterial, anthelmintic, and antineoplastic activities (Akinpelu et al., 2011). While saponins are surface-active agents which alter the permeability of the cell wall of organisms thus facilitating the entry of toxic materials or leakage of vital constituents from the cell (Daniyan et al., 2010), tannins were reported to exhibit antibacterial, antiviral and antitumor activities (Evans, 2002; Kolodziej and Kiderlen, 2005) and alkaloids are reported to possess antibacterial, anti-arrhythmics effect, anti-inflammatory and anti-asthmatic activity (Daniyan and Abalaka, 2012). The superior antioxidant and antimicrobial activity of the standard drugs compared to the endophytic fungus crude extract could be attributed to them containing more purified compounds than the extract (Ogbiko et al., 2018).

CONCLUSION

The study demonstrated that Curvulariaspp – an isolated endophytic fungus of Piliostigmathonningiileaf produce secondary metabolites with biological properties. These endophytic fungi can be a good source of novel therapeutic compounds, which may play a vital role in the development of antioxidant and antibacterial drugs for the effective treatment of free radical implicated disorders and selected microbial actions. Studies are still ongoing by the authors to isolate and characterize the particular compound(s) that is/are involved in the antioxidant and antimicrobial activities, and ascertain their potency in vivo.

CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest with the contents of this article.

REFERENCES

Akinpelu, A.D., Awotorebo, T.O., Agunbiade, O.M., Ayyegoro, A.O. and Okoh, I.A. (2011).Anti-vibrio and preliminary phytochemical characteristics of crude methanolic extracts of the leaves of Dialium guineense (Wild), Journal of Medicinal Plant Research, 5(11), 2398-2404.

Akpotu, M.O., Eze, P.M., Abba, C.C., Nwachukwu, C.U., Okoye, F.B.C. and Esimone, C.O. (2017)a. Metabolites of endophytic fungi Isolated from Euphorbia hirtagrowing in Southern Nigeria, Chemical Science Review and Letters, 6(21): 12 – 19.

Akpotu, M.O., Eze, P.M., Abba, C.C., Umeokoli, B.O., Nwachukwu, C.U., Okoye, F.B.C., Esimone, C.O. (2017)b. Antimicrobial activities of secondary metabolites of endophytic fungi isolated from Catharanthus roseus, Journal of Health Science, 7(1): 15-22.

Chika, C.A., Peter M.E., Dominic, O.A., Charles, U.N., Peter, P., Okoye, F.B.C. and Eboka, C.J. (2018).Phenolic Compounds from Endophytic Pseudofusicoccumsp. Isolated from Annona muricata. Tropical Journal of Natural Product Research, 2(7):332-337.

Cook, N.C. and Samman, S. (1996). Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources. Nutri Biochem, 7: 66-76.

Daniyan, S.Y. and Abalaka, M.E. (2012).Antimicrobial activity of stem extracts of Piliostigma thonningii, Journal of Science and Multidisciplinary Research, 1(2), 8-13.

Daniyan, S.Y., Galadima, M., Ijah, U.J.J., Odama, L.E., Yusuf, A.Y. and Abbas, Y. (2010).In vitro antibacterial screening of Piliostigma thonningii (Schum) Milne Red-Head leaves extracts against clinical isolates of methicillin resistant and methicillin sensitive Staphylococcus aureus, International Journal of Pure and Applied Sciences, 4(1), 88-94.

Deepa, M. and Renuka, D. (2014). Potent anti-inflammatory medical plant-a review, Int J Pharm Pharm Sci., 6(4):43-49.

Ebrahimzadeh, M.A., Pourmorad, F., Bekhradnia, A.R. (2008).Iron chelating activity, phenol and flavonoid content of some medicinal plants from Iran, Afr. J. Biotech, 7, 3188-3192.

Evans, W.C. (2002). Trease and Evans Pharmacognosy.Elsiever India, 15th edition. 27(46), 183-184.

Ezeja, M.I., Yusuf, N., Omeh, S., Onoja, O. and Ijeoma, H.U. (2015). Antiinflammatory and antioxidant activities of the methanolic leaf extract of Cissus aralioides, Am J Pharmacol Sci, 3 (1): 1-6.

Farrukh, A., Iqbal, A. and Zafar, M. (2006). Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. Turk. J. Biol, 30: 177-183.

Gandía-Herrero, F., Escribano, J. and García-Carmona, F. (2009).The role of phenolic hydroxy groups in the free radical scavenging activity of betalains, Journal of Natural Products, 72, 1142–1146.

Harman, D. (1981). The aging process. Proc, Natl Acad. Sci, USA. 78: 7124-7128

Jain, A., Soni, M., Deb, L., Jain, A., Rout, S., Gupta, V. and Krishna, K. (2008). Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of Momordica dioica leaves, J. Ethnopharmacol, 115, 61-66.

Khandewal, K.R. (2000). Practical Pharmacognosy Technique and Experiments, 9th edn,Nirali Publications Pune, pp. 149-156.

Kim, D.O., Jeong, S.W. and Lee, C.Y. (2003).Antioxidant capacity of phenolic phytochemicals from various cultivars of plums, Food Chem, 81, 321-326.

Kolodziej, H. and Kiderlen, A.F. (2005).Antileishmanial activity and immune modulatory effects of tannins and related compounds on Leishmania parasitised RAW 264.7 cells, Journal of Phytochemistry, 66(17), 2056-2071.

Kumpulainen, J.T. and Salonen, J.T. (1999). Natural Antioxidants and Anticarcinogens in Nutrition, Health and Disease, The Royal Society of Chemistry, UK. pp 178-187.

Kwaji, A., Bassi, P.U., Aoill, M., Nneji, C.M. and Ademowo, G. (2010).Preliminary studies on PiliostigmathonningiiSchumleaf extract: Phytochemical screening and in vitro antimalarial activity. African Journal of Microbiology Research, 4(9): 735-739.

Maisuthisakul, P., Pasuk, S. and Ritthiruangdej, P. (2008).Relationship between antioxidant properties and chemical composition of some Thai plants, Journal of Food Composition and Analysis, 21(3), 229-240.

Maisuthisakul, P., Pongsawatmanit, R. and Gordon, M.H. (2007).Assessment of phenolic content and free-radical scavenging capacity of some Thai indigenous plants, Food Chemistry, 100, 1409-1418.

Ogbiko, C., Eboka, C.J. and Tambuwal, A.D. (2018).Extraction and fractionation of whole black seed plantain (Plantago rugelii Decne) for in-vitro antioxidant, antibacterial and phytochemical screening, Journal of Applied Sciences and Environmental Management, 22(5), 613– 618.

Ogbiko, C., Okoye, F.B.C. and Eboka C.J. (2020).Phytochemical, in vitro antimicrobial, antioxidant properties and polyphenolic content of methanol leaves extract of Piliostigma thonningii Schum Milne-Redhead (Fabaceae). African Journal of Pharmaceutical Research and Development. 12(3): 299 – 312.

Okoye, F.B.C., Nworu, C.S., Debbab, A., Esimone, C.O. and Proksch, P. (2015).Two new Cytochalasins from an endophytic fungus, KL-1.1 isolated from Psidium guajava leaves. Phytochem Lett, 14:51-55.

Okoye, F. B. C. and Osadebe, P. O.(2010). 'A new anti-inflammatory flavonol glycoside from Alchornea floribunda leaves', Natural Product Research, 24(3): 266 – 273.

Vijay, P. and Vimukta, S. (2014). The role of natural antioxidants in oxidative stress induced diabetes mellitus, Research Journal of Pharmaceutical Sciences, 3, 1-6.

Yadav, M., Yadav, A. and Yadav, J.P. (2014).In vitro antioxidant activity and total phenolic content of endophytic fungi isolated from Eugenia jambolana Lam, Asian Pacific Journal of Tropical Biomedicine, 7(suppl. 1): 5256 – 5261.

Zhang, L. and Lin, Y. (2008).Tannins from Canarium album with potent antioxidant activity, Journal of Zhejiang University Science, 9(5), 407-415.

Zhang, Y.J., Gan, R.Y., Li, S., Zhou, Y., Li, A.N. and Xu, D.P. (2015). Antioxidant phytochemicals for the prevention and treatment of chronic diseases, Molecules, 20(12): 21138–21156.