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Abstract

Ocimum americanum L. (Lamiaceae) is a common food condiment and also used in traditional medicine in the management of several human diseases. Nonetheless, there has been no effort to delineate the biological and phytochemical profiles of leaves and flowers prepared by different extractive solvents (ethyl acetate, methanol (MeOH), and water). The pharmacological potential of *O. americanum* extracts on pro-oxidant/pro-inflammatory mediators in rat colon specimens treated with lipopolysaccharide was investigated. In parallel, the inhibitory effects of the extracts on fungal and bacterial strains involved in ulcerative colitis were studied. Qualitative phytochemical analysis showed the presence of phenols, flavonoids, and tannins. Water extracts of flowers and leaves showed strong reducing and radicals scavenging potential. Both MeOH and ethyl acetate extracts of the leaves and flowers were able to inhibit acetylcholinesterase, butyrylcholinesterase, and tyrosinase. All the extracts inhibited the selected bacterial and fungal strains, while only ethyl acetate flower extract displayed antioxidant/anti-inflammatory effects in rat colon. The water and MeOH extracts stimulated colon lactate dehydrogenase (LDH) and serotonin (5-HT) and induced spontaneous migration of HCT116 cells. Future investigations should focus on the biological activity of isolated phytochemicals from the leaves and flowers of *O. americanum*, in order to clarify the mechanism(s) of action substantiating the observed pharmacological properties.

Keywords: serotonin; anti-inflammatory; lactate dehydrogenase; free radicals; phenolic

Introduction

Ocimum americanum L. (Lamiaceae), also known as American basil or 'Hoary basil' forms part of the *Ocimum* genus, which comprises of more than 150 species distributed throughout temperate regions of the globe (Dzoyem, McGaw, Kuete, & Bakowsky, 2017). The aromatic leaves of *O. americanum* can be used as condiment (Aluko, Oloyede, & Afolayan 2013). For instance, it used in the preparation of soups (Bassole, Nebie, Savadogo, Ouattara, Barro, & Traore 2005), while the seeds can be used to prepare refreshing drinks (Upadhyay, Misra, & Singh, 1991). In traditional medicine, the leaf juice of *O. americanum* is used to manage dysentery, toothache, and migraine (Ekundayo, Laakso, & Hiltunen, 1989; Sen & Behera 2008; Sunitha & Begum, 2013; Silva et al., 2015). A decoction of the leaf has been documented to be used against nasal bleeding and malarial fever (Sunitha & Begum, 2013; Shah & Rahim, 2017). A paste obtained from the leaves was also used against skin diseases of parasitic origin (Leffers, 2003). On the other hand, an infusion prepared from the leaves was used to treat fever, indigestion, and diarrhoea (Aluko et al., 2013; Oyedemi et al., 2017). *O. americanum* is also used for the management of cold, coughs, cataract, and bronchitis (Sunitha & Begum, 2013; Pandey, Chandra, Kumar, Dutt, & Sharma, 2016; Oyedemi et al., 2017). Pharmacological evidences have shown that the crude methanol extract of *O. americanum* leaf exhibited synergistic interaction with norfloxacin against *Staphylococcus aureus* strains and showed non-toxic effect on the HepG2 cells (IC_{50} value 378.0 μg/mL) (Oyedemi et al., 2017). *O. americanum* leaf methanol extract has been reported to be an effective larvicidal and repellent agent against malarial and dengue vectors (Madhiyazhagan, Murugan, Kumar, & Nataraj, 2014). Essential oil obtained from fresh *O. americanum* leaves by hydrodistillation showed antioxidant and cytotoxic (breast cancer cell line, MCF-7) activities (Tamil Selvi,

Thirugnanasampandan, & Sundarammal, 2015). Diabetic and non-diabetic C57BL/KsJ mice administered with *O. americanum* aqueous extract showed reduction in fasting blood glucose levels and body weight (Nyarko, Asare-Anane, Ofosuhene, & Addy, 2002). The aqueous extract has been reported to reduce LDL and VLDL and increase antioxidant enzymes (catalase and superoxide dismutase) (Dash, Mishra, & Dash, 2014). Rosmarinic acid and caffeic acid derivatives were identified from the aqueous extract of *O. americanum* leaves (Berhow, Affum, & Gyan, 2012). It was reported that both compounds possess effective biological activities including antioxidant and enzyme inhibitory activity (Topal and Gulçin, 2014; Gülçin et al., 2016; Adomako-Bonsu et al., 2017). Volatile oils, flavonoids, phytosterols, and tannins have also been reported from *O. americanum* (Sarma and Babu, 2011).

It has been argued that he recovery of bioactive compounds from plant matrix depend on the extraction technique and type of solvent used. The nature and amount of secondary metabolites extracted from plant materials is greatly determined by the solvent used (Dirar et al., 2019). As such, several studies have reported activity of different solvents on the secondary metabolite extraction from plants and subsequent isolation of bioactive compounds. The selection of a suitable extraction method and solvent are key factors in the process of standardization of herbal products and to determine upscaling possibilities, i.e., from laboratory scale to pilot plant (Dirar et al., 2019). The majority of the studies conducted on *O. americanum* focused on the leaves of the plant and the essential oil extracted from this plant part. Currently, there is a paucity of literature concerning the effect of different extraction solvents on the phytochemical profiles and biological activities (antioxidant and enzyme inhibitory activities) of leaves and flowers extracts of *O. americanum*. However, the potential influence of extraction solvent on antioxidant capacity has been investigated for other *Ocimum* species (Gülçin et al., 2007). Thus, the main aim of the present study was to assess the effect of different extraction solvents (ethyl acetate, methanol, and water) on the phytochemical extraction and biological

activities of *O. americanum* leaves and flowers. The effect of *O. americanum* extracts on pro-oxidant and pro-inflammatory mediators (nitrites, lactate dehydrogenase, and serotonin) in ulcerative colitis experimental model of *ex vivo* rat colon specimens treated with lipopolysaccharide was also investigated. Additionally, the inhibitory effect of *O. americanum* extracts on fungal and bacterial species involved in ulcerative colitis, namely, *Candida albicans, C. tropicalis, Staphylococcus aureus*, and *Salmonella enterica* subsp. *enterica* serovar Typhimurium was determined.

2. Materials and Methods

2.1 Plant material and preparation of extracts

Ocimum americanum was collected in Ivory Coast (National Center for Agricultural Research Station, Divo Department, Lôh Djiboua Region). The botanical authentication was carried out by the botanist Dr. Diane Estelle Gnapi (Université Félix Houphouet Boigny, Abidjan, Ivory Coast). The flowers and leaves were collected and shade dried for 10 days at room temperature. The dried plant materials were then milled using a laboratory mill.

Maceration using methanol and ethyl acetate was carried out as follows: 5 g dried powdered plant sample was mixed with 100 mL of each solvent for 24 h. The extracts were then filtered and evaporated *in vacuo* at 40 °C using a rotary evaporator. On the other hand, the water extract was prepared following traditional infusion method, 5 g dried powdered plant sample was infused in 100 mL of boiling water for 20 min. The infusion was filtered and the filtrate was lyophilised. All extracts were stored at +4°C away from light until analysis.

2.2. Profile of bioactive compounds

By referring to our previous paper (Uysal et al., 2017; Zengin and Aktumsek, 2014), the flavonoids (TFC, expressed as rutin equivalent: mg RE/g), total phenolic (TPC, expressed as gallic acid equivalent: mg GAE/g), total phenolic acid (TPaC, expressed as caffeic acid

178 equivalent: mg CAE/g), total flavanol (TFvlC, expressed as catechin equivalent: mg CE/g),
179 total tannin (TTC, expressed as catechin equivalent: mg CE/g) and total saponin (TSC,
180 expressed as quillaja equivalent: mg QE/g) contents were determined.

181 A Dionex Ultimate 3000RS UHPLC instrument equipped with a Thermo Accucore C_{18}
182 column (100 mm x 2.1 mm and 2.6 μm particle size), kept at 25 °C (± 1 °C), was used for
183 secondary metabolites analysis. Gradient method was used to separate components and the
184 detailed protocol was reported in our previous article (Zengin et al., 2018).

185 *2.3. Evaluation of antioxidant and enzyme inhibitory effects*

186 The *in vitro* enzyme inhibitory effects of *O. americanum* extracts on α-amylase, α-
187 glucosidase, acetylcholinesterase (AChE), butyrylcholinestase (BChE), and tyrosinase were
188 evaluated, as previously reported (Uysal et al., 2017). The enzyme inhibitory actions of *O.*
189 *americanum* extracts were reported as equivalents of kojic acid (KAE) for tyrosinase,
190 galantamine for AChE and BChE, and acarbose for α-amylase and α-glucosidase. Regarding
191 antioxidant capacity of the extracts, different spectrophotometric experiments as ferrous ion
192 chelating, phosphomolybdenum, reducing power (FRAP and CUPRAC) and radicals
193 scavenging assays (ABTS and DPPH) were performed as previously reported (Uysal et al.,
194 2017). The results were expressed as equivalents of EDTA or Trolox (mg EDTAE/g and mmol
195 TE/g).

196 MixOmics R package and Xlstat 2017 softwares were used for statistical analysis.
197 Firstly, all data sets were uploaded into the R 3.5.1 software for multivariate analysis to identify
198 the major source of variation in dataset. Afterwards, the most discriminating factor was
199 considered and a further analysis was performed in order to obtain circos plot. The relationship
200 between the bioactive compounds and studied biological activities resulting from correlation
201 coefficient was then evaluated ($r = 0.70$). Finally, data were analysed using Two-way ANOVA

to investigate differences between the samples. $p<0.05$ was considered as statistically significant and Tukey's multiple range was done.

2.4. Pharmacological studies

2.4.1. Artemia salina lethality bioassay

Artemia salina Leach lethality bioassay was performed as previously reported (Ferrante et al., 2019). Briefly, brine shrimp larvae were incubated in the medium at 25-28 °C for 24 h in presence of *O. americanum* extracts ranging from 0.1 to 20 mg/mL. The number of dead and alive shrimps was counted after 24 h treatment with and without (control experiment) *O. americanum* extracts. Experiments were performed in triplicate, and percentage of mortality was determined as follows: $\frac{(T-A)}{T} \times 100$, where, T and S are the total numbers of incubated larvae and alive shrimps, respectively.

2.4.2. In vitro studies

Human colon HCT116 cells were cultured in DMEM (Euroclone), as previously described (Ferrante et al., 2019). The effects of *O. americanum* extracts (10-1000 µg/mL) on HCT116 viability was assessed through 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test, while the effects of *O. americanum* extracts (100 µg/mL) on HCT116 cell migration were evaluated using the wound healing experimental paradigm (Ju, Kwak, Hao, & Yang, 2012). The detailed procedure related to calculation and analysis of scratch area was reported in a previous paper (Ferrante et al., 2019).

2.4.3. *Ex vivo studies*

Sprague-Dawley rat tissues were collected as previously described (Ferrante et al., 2019). Isolated colon specimens were incubated with 5% CO_2 at 37 °C for 4 h in RPMI buffer. Isolated colons were stimulated with bacterial LPS (10 µg/mL) and simultaneously treated with

O. americanum extracts (100 μg/mL). At the end of incubation period, tissue supernatants were collected for nitrite level determination (Zengin et al., 2017). On the other hand, isolated colons were explanted and tissue 5-HT (ng/mg wet tissue) was extracted as previously reported (Brunetti et al., 2014; Ferrante et al., 2016). Thereafter, neurotransmitter level was quantified by HPLC coupled coulometric detector (Brunetti et al., 2014). Lastly, spectrophotometric determination of LDH level was performed according to manufacturer's protocol (Sigma-Aldrich). Experiments were conducted in triplicate and expressed as percentage activity compared to vehicle-treated cells (Menghini et al., 2018).

2.5. Microbiological studies

Antibacterial and antifungal activity of *O. americanum* extracts was studied against *Staphylococcus aureus* (ATCC 6538), *Salmonella* Thyphimurium (clinical isolate), *Candida albicans* (YEPGA 6183), and *C. tropicalis* (YEPGA 6184). The experimental conditions for microbiological tests were carried out as recently described (Ferrante et al., 2019).

Data gathered from the pharmacological assays were expressed ad means ± S.E.M. and analyzed by one-way analysis of variance (ANOVA), followed by Newman-Keuls comparison multiple test (GraphPad Prism version 5.01 for Windows). Results were considered significant for p values less than 0.05. While the number of animals was determined using of the "Resource Equation" $N=(E+T)/T$ $(10 \leq E \leq 20)$ (Charan & Kantharia, 2013).

3. Results

Phytochemical profile

In the current study, the total phenolic, flavonoid, flavanol, phenolic acid, and tannin contents of the extracts were determined to assess the impact of different extraction solvents. Total phenolic content ranged from 50.68 to 147.94 mg GAE/g. The highest concentration of phenolic was found in the water extracts. However, the phenolic content of water extract of *O. americanum* flower was significantly ($p<0.05$) higher than the water extract of *O. americanum*

leaves. A similar trend was observed for total phenolic acid estimations, i.e., in general the total phenolic acid content of water extracts was higher. Similarly, it was noted that the total phenolic acid content of the water extract of *O. americanum* flower was significantly ($p<0.05$) higher than the water extract of *O. americanum* leaves. The flavonoid content of the extracts was also measured and presented in Table 1. Methanol was observed to be a good extraction solvent for flavonoids and *O. americanum* flower methanol extract (37.20 mg RE/g) showed high flavonoid content. Compared to the other solvents, ethyl acetate extracts showed higher flavanol (11.89 and 8.30 mg CE/g, for flower and leaves extracts, respectively) and tannin (5.88 and 11.36 mg CE/g, for flower and leaves extracts, respectively) contents. Ethyl acetate extract of *O. americanum* leaves had the highest concentration of saponins (206.58 mg QE/mg).

The identity of the phytoconstituent was based on comparison with the detected mass, fragmentation obtained from standards and literature data. For establishment of full phenolic pattern of *O. americanum*, the methanol, ethyl acetate, and water extracts of flowers and leaves were analysed using LC-MS/MS. Forty-five compounds were identified in the methanol extract of the flower, 41 compounds in the water extract and 39 compounds in the ethyl acetate extract. The results were presented in Table 2. The major compounds belong to flavonoid and polyphenol groups. Caftaric acid, gallic acid, N-trans-feruloyltyramine, and quercetin-di-O-hexoside were identified in the methanol and water extracts of the flowers; nevadensin, pilosin, and salvigenin in the methanol and ethyl acetate of *O. americanum* flowers. Additionally, luteolin-O-feruloylhexoside, tuberonic acid and its hexoside were present in the methanol extract, while riboflavin and verbascoside isomer were present in the water extract. The presence of luteolin, cirsiliol, apigenin, cirsimaritin, cirsilineol, xanthomicrol, and genkwanin in *O. americanum* was reported in a previous study (Vieira, Grayer, & Paton, 2003). The details of chemical profiles of the tested extracts are provided in Supplemental material.

In the leaf extracts, 59 compounds were identified in the methanol extract, 38 compounds in the water extract, and 34 compounds in ethyl acetate extract. Dihydroxy-tetramethoxy(iso)flavone, dodecanedioic acid, eriodictyol, feruloylhexose isomers, feruloylhexose isomer 2, isoquercitrin, and jasmonic acid were identified in the methanol, ethyl acetate, and water extracts of *O. americanum* leaves.

Fatty acids, such as stearidonic, α-linolenic, linoleic acid, palmitic, oleic, stearic, and arachidic acids were detected from the methanol and ethyl acetate extracts of *O. americanum* leaves. Previous studies have reported the presence of these fatty acids in seed oil of *O. basilicum, O. canum, O. gratissimum* (Angers, Morales, & Simon, 1996), and leaves of *O. sanctum* (Vidhani et al., 2016).

Antioxidant activities

Antioxidant activities of the methanol, ethyl acetate, and water extracts of *O. americanum* flowers and leaves as determined by $ABTS^{\bullet+}$, $DPPH^{\bullet}$, CUPRAC, FRAP, metal chelating, and phosphomolybdenum are shown in Table 3. The water extracts showed higher radical scavenging properties. Quantitative phytochemical studies revealed that water extracts were rich in phenolics and phenolic acids. A similar trend was observed for reducing assays. As compared to the other extracts, water extracts of *O. americanum* flowers (654.64 and 453.50 mg TE/g for CUPRAC and FRAP assays, respectively) and leaves (494.56 and 342.39 mg TE/g for CUPRAC and FRAP assays, respectively) showed strong reducing potential. In the presence of antioxidant compounds such as phenolics and phenolic acids, TPTZ-Fe^{3+} complex is reduced to TPTZ-Fe^{2+} complex, producing a chromophore having maximum absorption at 593 nm and neocuproine-Cu^{2+} complex is reduced to neocuproine-Cu^{+} complex, producing an orange yellow chromophore having maximum absorption at 450 nm. A different pattern was observed regarding metal chelating capability. From Table 3, it was noted that the ethyl acetate extract of *O. americanum* leaves, having highest tannin content, showed significant metal chelating

properties. Krzyzowska et al. (2017) claimed that tannins can act as metal chelating agents. Phiwchai, Yuensook, Sawaengsiriphon, Krungchanuchat, and Pilapong (2018) observed the extracellular iron binding of tannic acid resulting in the formation of self-assembled Fe^{3+}-tannic acid complexes. Additionally, tannic acid was found to inhibit iron-induced HepG2 cell growth.

Enzyme inhibitory activities

The inhibitory activities of the different extracts of *O. americanum* flowers and leaves on α-amylase, α-glucosidase, AChE, BChE, and tyrosinase are summarised in Table 4. Evaluation of the inhibitory activity of the extracts against enzymes related to diabetes mellitus type 2 was reported as acarbose equivalent, since acarbose is a clinically used oral hypoglycemic drug. Inhibitory activity against α-amylase ranged from 0.15 to 0.79 mmol ACAE/g while a higher range was observed against α-glucosidase (from 1.44 to 12.76 mmol ACAE/g). From Table 4, it was noted that the ethyl acetate and methanol extracts of the flowers and leaves of *O. americanum* were active inhibitors of both AChE and BChE. Interestingly, *O. americanum* extracts showed pronounced inhibitory action against tyrosinase, as well.

Identification of the major source of variation in dataset

The extracting solvent and the plant part may constitute a probable source of variation, which can explain the observed difference in total bioactive compounds and antioxidants activities, as well as enzyme inhibitory activities between the extracts. In order to investigate the effects of extracting solvent and plant part, a multi-block analysis with DIABLO was applied to the dataset. DIABLO is an integrative method that extends PLS models and uses both sGCCA (sparse Generalized Canonical Correlation Analysis) and sPLS-DA (sparse PLS-Discriminant Analysis) to a supervised analysis framework and multi-omics analyses, respectively (Lê Cao, Boitard, & Besse, 2011; Tenenhaus et al., 2014; Wold, 1966).

The plots of samples from each dataset built individually, by using the extracting solvent and plant part as class membership criteria respectively are provided in Figure 1 A&B. As

observed in the different plots, as opposed to result obtained with the plant parts as factor, a clear separation of samples was achieved with the second factor e.g. the extracting solvent. Accordingly, the extracting solvent was the only influence factor accountable for the observed variation of the bioactive compound content, antioxidant abilities as well as enzymes inhibitory activities of *O. americanum* extracts.

The change of polarity of solvents tend to influence the efficiency of bioactive compounds extraction, thereby affecting the biological activities. As previously observed, each solvent gave distinct specificities in the extraction of bioactive compounds. Phenolic content and phenolic acid were better extracted by water, the most polar solvent used in this study. Methanol, a slightly more polar solvent, was suitable for the extraction of flavonoids whereas the less polar solvent, ethyl acetate, effectively extracted flavanols, saponins, and tannins. Through this observation, it seems very complex to use a standard extraction solvent for the extraction of all active compounds. Therefore, the solvent for extraction must be determined according to the nature of interested active biomolecules. Otherwise, it is worth noting the similarity between the methanol and ethyl acetate extracts. As we have seen in clustered image map (Figure 1D) based on the three datasets, the extracts obtained with these two solvents showed some relatively similar biological activities and bioactive compounds content.

The prediction performance of the two studied models was assessed by estimating BER (classification error rate), using “max. dist” as prediction distance and 5-fold CV repeated 10 times. As depicted in Figure 1C, the best performance was achieved for 4 and 2 components, respectively. Much more, the confusion table comparing our dataset with the samples from a test dataset, indicated that the obtained 4 and 2 components of respective models were enough (Figure 2A).

A global overview of the correlation between total bioactive compounds, antioxidant activities and enzymes inhibitory activities datasets were represented in Figure 2B. A strong

positive correlation was achieved between total bioactive compounds and antioxidant activities datasets (r = 0.99); as well as between total bioactive compounds and enzymes inhibitory activities datasets (r = 0.89). An in-depth analysis, revealed a significantly positively link between TPC, predominantly total phenolic acid content (TPaC) and antioxidant assays (ABTS, DPPH, FRAP, CUPRAC) (Figure 2C), indicating that TPaC was associated with the antioxidant activity observed in *O. americanum*. In addition, the contribution of TFC in the AChE inhibition was highlighted, while a synergetic effect of total saponin content, total tannin content, and total flavanol content (TFvlC) in the BChE inhibition was observed (Figure 2C).

Pharmacological studies

The potential toxicity of the methanol, ethyl acetate, and water extracts of *O. americanum* leaves and flowers (0.1-20 mg/mL) was evaluated on brine shrimp (*A. salina*). After treatment with *O. americanum* extracts, the LC_{50} values were > 1.05 mg/mL.

The antioxidant/antinflammatory effects of methanol, ethyl acetate, and water extracts of *O. americanum* leaves and flowers (100 µg/mL) on isolated rat colon challenged with LPS were studied. Particularly, blunting effects on LPS-induced levels of biomarkers of nitrosative stress, including nitrites (Figure 3) were observed. Only the methanol extract of *O. americanum* leaves potentiated LPS-induced production of nitrites. As regards LPS-induced LDH level, only the ethyl acetate extract of *O. americanum* flowers displayed inhibitory effect (Figure 4), whereas all other extracts potentiated LPS effect. The evaluation of 5-HT level, after challenging isolated rat colon with LPS, showed that all flower extracts and leaf ethyl acetate extract were able to counteract LPS stimulating effect on tissue 5-HT concentration (Figure 5). Conversely, methanol and water leaf extracts increased 5-HT levels in rat colon specimens challenged with LPS.

The effect of the extracts on human colon cancer HCT116 cell line was also studied. Only the ethyl acetate leaf extract inhibited cell viability in the MTT test (Figure 6). Conversely,

379 methanol and water leaf extracts were able to stimulate HCT116 cell spontaneous migration
380 (Figure 7) in wound healing experimental paradigm.

381 Finally, the inhibitory effects of methanol, ethyl acetate, and water extracts of *O.*
382 *americanum* leaves and flowers on selected pathogen bacterial and fungi strains were studied.
383 The findings highlighted significant inhibitory effects on *S. aureus* and *S.* Typhimurium (Table
384 5), and *C. albicans* and *C. tropicalis* (Table 6). The MIC values related to antibacterial and
385 antifungal effects were comparable with those of ciprofloxacin and fluconazole, respectively.
386

387 **4. Discussion**

388 Secondary metabolites are ubiquitous in medicinal food plants and present several
389 health benefits including antioxidant properties. Phenolics and phenolic acids neutralize
390 $ABTS^{\bullet+}$ and $DPPH^{\bullet}$ either by direct reduction *via* electron transfer or by radical quenching *via*
391 hydrogen atom transfer (Cerretani & Bendini, 2010). Krzyzowska et al. (2017) also claimed
392 that tannins can act as metal chelating agents. While Phiwchai, Yuensook, Sawaengsiriphon,
393 Krungchanuchat, and Pilapong (2018) observed the extracellular iron binding of tannic acid
394 resulting in the formation of self-assembled Fe^{3+}-tannic acid complexes. The higher
395 concentration of phenolics found in the water extracts, compared to more lipophilic extracts,
396 are consistent, albeit partially, with the reported radical scavenging properties (Table 3). It is
397 also noteworthy highlighting that secondary metabolites can deliver excellent pharmacophore
398 patterns as enzymatic inhibitors for drug development for the management of type 2 diabetes,
399 Alzheimer's disease, and epidermal hyperpigmentation problems. It was noted that a
400 particularly high α-glucosidase inhibition was recorded for the ethyl acetate extract of *O.*
401 *americanum* leaves. Quantitative phytochemical analysis showed the high concentration of
402 tannins present in *O. americanum* leaves ethyl acetate extracts. This finding is supported by
403 several reports which have described the inhibitory action of some tannins on α-glucosidase

(Barrett, Farhadi, & Smith, 2018; Lee, Kim, Yang, & Sung, 2017; Omar, Li, Yuan, & Seeram, 2012). The efficacy of *O. americanum* extracts in inhibiting cholinesterases was also assessed. Cholinesterases inhibitors are regarded as valid therapeutic targets for the management of Alzheimer's disease. Galantamine, a phenanthrene alkaloid of plant origin, possesses neuroprotectant abilities as indicated by its capacity to inhibit cholinesterase enzymes (Y. Zhang, Zhao, Wu, Chen, & Ma, 2017). However, reports of the Australian Adverse Drug Reaction Advisory Committee described 18 cases of delirium/confusion, 8 of syncope, 13 of bradycardia, 6 of other dysrhythmias/conduction abnormalities, and 6 of hypotension, observed in Alzheimer's patients treated with galantamine, highlighting a dire need for novel compounds (Aagaard, 2014). There is a widespread consensus that AChE is a key enzyme regulating neurotransmission in cholinergic pathways and is an important target in the treatment of neurodegenerative disorders, such as Alzheimer's disease (Boztas et al., 2019; Atmaka et al., 2019; Genç Bilgiçli et al., 2019). In contrast to the long-established and well-defined role of AChE in the regulation of cholinergic transmission, the accurate physiological role of BChE remains elusive (Pope & Brimijoin, 2018). Studies have shown that BChE activity increased in the late stage of Alzheimer's disease. The interaction of rosmarinic acid, identified in the ethyl acetate and methanol extracts of *O. americanum* leaves and flowers, has been previously described. The hydroxyl groups rosmarinic acid interacted to the active site of BChE by forming hydrogen bonds with TRP82 and TYR128 residues (Senol et al., 2017). High binding affinity was also observed with AChE (Demirezer et al., 2015). Quercetin and its glycosylated form, rutin, identified in the active extracts, demonstrated inhibitory action against both cholinesterase enzymes (Ademosun, Oboh, Bello, & Ayeni, 2016). The inhibitory activity observed against AChE and BChE might be related to the concerted action of different compounds. Regarding the inhibitory activity against tyrosinase, a significant inhibitory effect was recorded for methanol and ethyl acetate extracts of *O. americanum* leaves and flowers.

Tyrosinase inhibition has attracted considerable attention in the management of epidermal hyperpigmentation problems but is also a new target in the management of neurodegenerative complications such as Parkinson's disease. Tyrosinase is a copper-containing enzyme crucial in the biosynthesis of melanin, a brown pigment which protects the human skin from UV radiation (R. Wang, Wang, Xia, Sui, & Si, 2019). However, an excessive production and accumulation of melanin favours the development of epidermal hyperpigmentation problems and dermatological conditions. Additionally, of late tyrosinase has been related to dopamine neurotoxicity and neurodegeneration, thereby associating tyrosinase over activity to Parkinson's and other neurodegenerative diseases (Hu et al., 2019). Previous docking studies have shown that quercetin (identified in *O. americanum* leaves and flowers extracts) interacted with the active site of tyrosinase and chelated copper with the 3', 4'-dihydroxy groups, thus preventing the binding of substrate. Besides, rosmarinic acid has also been reported to inhibit tyrosinase (Fan, Zhang, Hu, Xu, & Gong, 2017; Kang, Kim, Byun, Park, & Cho, 2004). The intricate synergism operating between the compounds could be responsible for the observed inhibition.

In the present study, a pharmaco-toxicological investigation was performed, as well, in order to confirm the intrinsic antioxidant effects, but also to investigate potential applications of *O. americanum* leaf and flower extracts, through a multidirectional approach, *in vitro*. Firstly, we explored the range of biocompatibility with a toxicological model constituted by brine shrimp (*A. salina*), which is commonly used to investigate a variety of biological and toxicological activities of plant extracts and is considered, at least partially, predictive of cytotoxicity (Ohikhena et al., 2016; Ferrante et al., 2019). The calculated LC_{50} was indicatory to select the extract concentration (100 μg/mL) for the subsequent biological studies.

Particularly, antioxidant/anti-inflammatory effects of *O. americanum* extracts were investigated on isolated rat colon fragments stimulated with LPS, an *ex vivo* paradigm reproducing the burden of inflammatory and oxidative stress occurring in ulcerative colitis

(Locatelli et al., 2017; Menghini et al., 2016; Menghini et al., 2018). Increased reactive oxygen (ROS) and nitrogen species (RNS) have long been related to tissue damage through the onset of disruptive peroxidation reactions of nucleic acids, proteins, and lipids (Uttara, Singh, Zamboni, & Mahajan, 2009; Gülçin, 2012). To this regard, lipid peroxidation plays a key role in ulcerative colitis (Achitei et al., 2013).The measurement of nitrite level is regarded as a tool to evaluate nitrosative stress, which is strictly related to lipid peroxidation, particularly in ulcerative colitis (Goggins et al., 2001).

It was observed that all *O. americanum* extracts, except the leaf methanol extract, were able to blunt the upregulation of colon nitrite level induced by LPS (Figure 3). Regarding LDH level assessment, experimental data showed that most of the *O. americanum* extracts were ineffective. Additionally, extract supplementation in tissue medium enhanced LPS-induced LDH level in isolate colon (Figure 4). Only the ethyl acetate extract of *O. americanum* flower revealed able to blunt LPS-induced LDH, thus suggesting protective effects in the colon. LDH is a well-recognized marker of tissue damage, particularly in the colon. Multiple studies suggested that downregulation of LDH level could be protective mechanism in ulcerative colitis (Kannan & Guruvayoorappan, 2013; Nagarjun, Dhadde, Veerapur, Thippeswamy, & Chandakavathe, 2017).

We also investigated the blunting effect of *O. americanum* on upregulated levels of 5-HT, which plays a key role as pro-inflammatory agent in the colon. The role of 5-HT, as colon pro-inflammatory cytokine, has been previously described (Regmi, Park, Ku, & Kim, 2014) and has been related to 5-HT3 receptor signaling (Mousavizadeh, Rahimian, Fakhfouri, Aslani, & Ghafourifar, 2009). In this context, LPS stimulus was able to increase steady state 5-HT level, in the colon, while antioxidant and anti-inflammatory extracts revealed effective in blunting 5-HT upregulation induced by inflammatory stimulus (Locatelli et al., 2017; Menghini et al., 2016). The evaluation of neurotransmitter tissue level has long been considered as a

reliable assessment of neurotransmitter synthesis and release (Bungo, Shiraishi, Yanagita, Ohta, & Fujita, 2009; Clark, MohanKumar, Kasturi, & MohanKumar, 2006). Concerning 5-HT level, flower extracts, together with ethyl acetate extract of *O. americanum* leaves inhibited LPS-induced 5-HT level (Figure 5). The blunting effects induced by these extracts on the selected biomarkers could be related to flavonoid-induced inhibitory effects on LDH, 5-HT3 receptor and i-NOS, whose upregulation has been causally related to inflammatory conditions and tissue damage (Chandel, Rawal, & Kaur, 2018; He et al., 2018; Herbrechter et al., 2015; Mousavizadeh et al., 2009; W. Zhang et al., 2018). On the other hand, water and methanol extract of *O. americanum* leaves, despite showing significant amounts of total phenols and flavonoids (Table 1), enhanced LPS-induced 5-HT level, thus indicating pro-inflammatory effects with potential tissue damages, as also suggested by the concomitant increase of LDH levels (Figure 4). Nevertheless, recent findings by Abnosi and Yari (2018) highlighted the potential toxicity of gallic acid in rat bone marrow mesenchymal stem cells, partially related to increased inflammatory and oxidative stress mediators, including LDH.

The effect of *O. americanum* extracts on viability (MTT test) and spontaneous migration (wound healing test) of human colon cancer HCT116 cell line was also investigated. Consistent with the inhibition of pro-oxidant and pro-inflammatory biomarker level in the colon, the antiproliferative effect induced by ethyl acetate extract of *O. americanum* flower confirmed its role as protective agent in rat colon (Figure 6). Conversely, the stimulation of HCT116 wound healing process could account for an increase in migration and invasion capacities of this cell line, thus further suggesting a potential toxicity induced by water and methanol extracts of *O. americanum* leaves (Figure 7). Considering that LDH may stimulate cell migration through mitochondrial ROS production (Valvona, Fillmore, Nunn, & Pilkington, 2016), increased HCT116 migration following treatment with water and methanol extract of *O. americanum* leaves could be related to the observed stimulation of LDH level in the colon.

Finally, the inhibitory effects of *O. americanum* extracts on bacteria and fungi, which potentially contribute in ulcerative colitis, have been studied (Cho & Chae, 2004; Guo et al., 2015; Iguidbashian, Parekh, Kukrety, & Andukuri, 2018; Trojanowska et al., 2010; X. Wang et al., 2018). *O. americanum* extracts were effective against both *S. aureus* and *S.* Typhimurium (Table 5). The same extracts also inhibited *C. albicans* and *C. tropicalis* (Table 6). Actually, the inhibitory effects exerted by *O. americanum* extracts on the selected bacteria and fungi strains is consistent with extract total content in phenols and flavonoids (Bottari et al., 2017; de Camargo et al., 2017). These findings, besides corroborating to the preliminary observations by Thaweboon and Thaweboon (2009) about antibacterial and antifungal effects of *O. americanum* essential oil, further claims that this species might be considered as potential and innovative source of antibacterial and antifungal compounds.

5. Conclusion

Scientific data collected from the present investigation supported that the leaves and flowers of *O. americanum* contain several classes of secondary metabolites which might be responsible for the observed biological activities. Our findings also highlighted the influence of solvent choice on the extraction yield of secondary metabolites from plants. The water extracts of the leaves and flowers of *O. americanum* demonstrated high radical scavenging and reducing activity. In general, the extracts of *O. americanum* were poor inhibitor of α-amylase and potent α-glucosidase inhibitor. The methanol and ethyl acetate extracts of *O. americanum* exhibited highest inhibition against acetylcholinesterase, butyrylcholinesterase, and tyrosinase. On the other hand, toxicological assays indicated a potential toxicity of the methanol and water extracts of *O. americanum* leaves. Conversely, ethyl acetate extracts displayed the best toxicological profile, exhibiting both anti-inflammatory and antiproliferative effects in the colon. All the extracts revealed to be effective antimicrobial agents, thus suggesting potential

applications in the isolation of antibacterial and antifungal compounds. Future investigations should focus on the determination of the biological activity of isolated phytochemicals from the leaves and flowers of *O. americanum,* in order to elucidate the mechanisms of action substantiating the observed effects.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Legends of Figures

Figure 1. Supervised multi datasets analysis with DIABLO, A&B: Sample plot with confidence ellipse plots based on the parts of plant and the extracting solvent as class membership criteria respectively, C: prediction model performance per component for Maximum Distance using 5-fold CV repeated 10 times, D: Clustered Image Map (Euclidean Distance, Ward linkage).

Figure 2. A: The confusion table for a 4 and 2 component respectively, B: Sample scatterplot displaying the global overview of the correlation between the three data sets at the two first component level, C: Circosplot shows the correlation the correlation (r = 0.70) between total bioactive compounds and evaluated biological activities.

Figure 3. Effect of water, methanol, and ethyl acetate extracts of *Ocimum americanum* leaves (O. Lea.) and flowers (O. FL.) (100 µg/mL) on LPS-induced nitrite level in rat colon specimens. ANOVA, $p<0.0001$; post-hoc, *$p<0.05$, ** $p<0.01$, *** $p<0.001$ *vs*. LPS.

Figure 4. Effect of water, methanol, and ethyl acetate extracts of *Ocimum americanum* leaves (O. Lea.) and flowers (O. FL.) (100 µg/mL) on LPS-induced LDH activity in rat colon specimens. ANOVA, $p<0.01$; post-hoc, * $p<0.05$ *vs*. LPS.

Figure 5. Effect of water, methanol, and ethyl acetate extracts of *Ocimum americanum* leaves (O. Lea.) and flowers (O. FL.) (100 µg/mL) on LPS-induced 5-HT level in rat colon specimens. ANOVA, $p<0.0001$; post-hoc*** $p<0.001$ *vs*. LPS.

Figure 6. Effect of water, methanol, and ethyl acetate extracts of *Ocimum americanum* leaves (O. Lea.) and flowers (O. FL.) (100 µg/mL) on tumoral HCT116 cell line viability (MTT test). ANOVA, $p<0.0001$; post-hoc, *** $p<0.001$ *vs*. CTR.

Figure 7. Effect of water, methanol, and ethyl acetate extracts of *Ocimum americanum* leaves (O. Lea.) and flowers (O. FL.) (100 µg/mL) on HCT116 migration (Wound healing test). ANOVA, $p<0.05$; post-hoc, *$p<0.05$ *vs*. respective CTR group.

Table 1. Total bioactive compounds in ethyl acetate, methanol, and water extracts of *Ocimum americanum* flowers and leaves.

Samples	Total phenolic content (mg GAE/g)	Total flavonoid content (mg RE/g)	Total flavanol content (mg CE/g)	Total phenolic acid content (mg CAE/g)	Total tannin content (mg CE/g)	Total saponin content (mg QE/g)
Flowers-EA	53.57±0.31^{e}	35.90±0.21^{b}	11.89±0.19^{a}	na	5.88±0.68^{b}	136.47±20.67^{b}
Flowers-MeOH	71.87±0.51^{c}	37.20±0.33^{a}	3.01±0.07^{c}	17.19±0.35^{c}	3.29±0.39^{c}	122.74±12.59^{b}
Flowers-Water	147.94±0.49^{a}	9.81±0.02^{c}	1.68±0.02^{e}	54.65±0.77^{a}	2.26±0.06^{cd}	72.74±1.69^{c}
Leaves-EA	57.72±0.52^{d}	6.61±0.98^{d}	8.30±0.01^{b}	na	11.36±0.73^{a}	206.58±16.73^{a}
Leaves-MeOH	50.68±0.29^{f}	34.82±0.13^{b}	2.73±0.05^{d}	11.97±0.29^{d}	2.80±0.04^{c}	143.23±13.16^{b}
Leaves-Water	94.25±0.75^{b}	6.25±0.05^{d}	1.42±0.02^{f}	31.10±0.90^{b}	1.32±0.12^{d}	110.15±1.88^{b}

* Values expressed are means ± S.D. of three parallel measurements. GAE: Gallic acid equivalent; RE: Rutin equivalent. CE: Catechin equivalent; CAE: Caffeic acid equivalent; QE: Quillaja equivalent. Different letters indicate significant differences in the tested extracts (p<0.05).

Table 2. LC MS/MS screening of ethyl acetate, methanol, and water extracts of *Ocimum americanum* flowers and leaves.

NO.	Name	$[M + H]^+$	$[M - H]^-$	Flowers-EA	Flowers-MeOH	Flowers-H_2O	Leaves-EA	Leaves-MeOH	Leaves-H_2O
1	Quinic acid		191.05557	+	+	+	+	+	+
2	Gallic acid		169.01370	-	+	+	-	+	+
3	Caftaric acid (2-O-Caffeoyltartaric acid)		311.04031	-	+	+	-	+	+
4	Fertaric acid (2-O-Feruloyltartaric acid)		325.05596	-	+	+	-	+	+
5	Chlorogenic acid (3-O-Caffeoylquinic acid)	355.10291		+	+	+	-	+	+
6	Caffeoylhexose		341.08726	+	+	+	-	+	-
7	Caffeic acid		179.03444	+	+	+	+	+	+
8	Coumaroylhexose isomer 1		325.09235	+	+	+	-	+	+
9	Coumaroylhexose isomer 2		325.09235	+	+	+	-	+	+
10	Feruloylhexose isomer 1		355.10291	-	-	-	+	+	+
11	Feruloylhexose		355.10291	+	+	+	-	-	-
12	Quercetin-di-O-hexoside		625.14048	-	+	+	-	-	-
13	Tuberonic acid hexoside		387.16551	+	+	+	+	+	+
14	4-Coumaric acid		163.03952	+	+	+	+	+	+
15	Feruloylhexose isomer 2		355.10291	-	-	-	+	+	+
16	Tuberonic acid		225.11269	+	+	+	-	+	+
17	Riboflavin	377.14611		-	-	+	-	+	+
18	Vicenin-2 (Apigenin-6,8-di-C-glucoside)		593.15065	+	+	+	-	+	+
19	Chicoric acid (2,3-Di-O-caffeoyltartaric acid)		473.07201	-	-	-	-	-	+
20	Eriodictyol-7-O-glucoside (Miscanthoside)		449.10839	+	+	+	-	+	+
21	Vitexin (Orientoside, Apigenin-8-C-glucoside)	433.11347		-	-	-	-	+	+
22	Quercetin-O-galloylhexoside		615.09862	-	-	-	-	+	+
23	Isovitexin (Apigenin-6-C-glucoside)	433.11347		-	-	-	-	+	+
24	Verbascoside		623.19760	+	+	+	-	+	-
25	Luteolin-7-O-glucoside (Cynaroside)		447.09274	+	+	+	+	+	+
26	Quercetin-O-glucuronide		477.06692	-	-	-	-	+	+
27	Ellagic acid		300.99845	-	-	-	-	+	-
28	Isoquercitrin (Hirsutrin, Quercetin-3-O-glucoside)		463.08765	+	+	+	+	+	+

29	Rosmarinic acid-O-hexoside		521.12952	+	+	+	-	+	-
30	N-trans-Feruloyltyramine	314.13924		-	-	+	-	+	-
31	3-O-Methylrosmarinic acid		373.09235	-	-	-	-	+	+
32	Rutin (Quercetin-3-O-rutinoside)	611.16122		+	+	+	-	+	+
33	Verbascoside isomer		623.19760	-	-	+	-	-	-
34	Cosmosiin (Apigetrin, Apigenin-7-O-glucoside)	433.11347		+	+	+	-	+	+
35	Methyl caffeate	195.06574		+	+	+	+	+	+
36	Rosmarinic acid (Labiatenic acid)		359.07670	+	+	+	+	+	+
37	Eriodictyol		287.05556	+	+	-	+	+	-
38	Methoxy-tetrahydroxy(iso)flavone-O-hexoside		477.10330	+	+	+	-	+	-
39	Luteolin-O-malonylhexoside isomer 1		533.09314	+	+	+	-	-	-
40	Luteolin-O-malonylhexoside isomer 2		533.09314	+	+	+	-	-	-
41	Quercetin-O-coumaroylhexose		609.12444	+	+	+	-	+	-
42	Methyl rosmarinate		373.09235	-	-	-	-	+	-
43	Quercetin		301.03483	+	+	+	-	+	-
44	Jasmonic acid		209.11777	+	+	+	+	+	+
45	Luteolin (3',4',5,7-Tetrahydroxyflavone)		285.03991	+	+	+	+	+	+
46	Luteolin-O-feruloylhexoside		623.14009	-	+	-	-	-	-
47	Di-O-methylellagic acid		329.02975	-	-	-	+	-	-
48	Cirsiliol (6,7-Dimethoxy-3',4',5-trihydroxyflavone)		329.06613	+	+	+	+	+	+
49	Apigenin (4',5,7-Trihydroxyflavone)		269.04500	+	+	+	+	+	+
50	Pilosin (4',6-Dimethoxy-5,7,8-trihydroxyflavone)		329.06613	+	+	-	+	+	-
51	Cirsimaritin (4',5-Dihydroxy-6,7-dimethoxyflavone)	315.08687		+	+	+	+	+	+
52	Cirsilineol (4',5-Dihydroxy-3',6,7-trimethoxyflavone)	345.09743		+	+	+	+	+	+
53	Xanthomicrol (4',5-Dihydroxy-6,7,8-trimethoxyflavone)	345.09743		+	+	+	+	+	+
54	Dihydroxy-tetramethoxy(iso)flavone		373.09235	+	+	+	+	+	+
55	Nevadensin (5,7-Dihydroxy-4',6,8-trimethoxyflavone)	345.09743		+	+	-	-	+	-
56	Caffeic acid phenethyl ester		283.09704	-	-	-	+	+	-
57	Genkwanin (4',5-Dihydroxy-7-methoxyflavone)	285.07630		+	+	+	+	+	-
58	Salvigenin (5-Hydroxy-4',6,7-trimethoxyflavone)	329.10251		+	+	-	-	-	-

59	Gardenin B (5-Hydroxy-4',6,7,8-tetramethoxyflavone)	359.11308		+	+	-	+	+	-
60	Dodecanedioic acid		229.14399	-	-	-	+	+	+
61	Stearidonic acid		275.20111	-	-	-	+	+	+
62	α-Linolenic acid		277.21676	-	-	-	+	+	-
63	α-Linolenic acid isomer		277.21676	-	-	-	+	-	-
64	Linoleic acid		279.23241	-	-	-	+	+	-
65	Palmitic acid		255.23241	-	-	-	+	+	-
66	Oleic acid		281.24806	-	-	-	+	+	-
67	Stearic acid		283.26371	-	-	-	+	+	-
68	Arachidic acid		311.29501	-	-	-	+	-	-

Table 3. Antioxidant properties of the ethyl acetate, methanol, and water extracts of *Ocimum americanum* flowers and leaves.

Samples	DPPH (mg TE/g)	ABTS (mg TE/g)	CUPRAC (mg TE/g)	FRAP (mg/TE)	Metal chelating (mg EDTAE/g)	Phosphomolybdenum (mmol TE/g)
Flowers-EA	19.75±0.63^{e}	63.73±3.00^{e}	123.51±5.79^{f}	55.60±0.81^{e}	9.87±0.81^{d}	2.07±0.17^{bc}
Flowers-MeOH	109.73±1.92^{c}	120.37±1.54^{c}	273.96±0.39^{c}	163.23±2.44^{c}	19.06±0.82^{b}	1.83±0.05^{c}
Flowers-Water	337.79±3.72^{a}	255.47±13.29^{a}	654.64±3.31^{a}	453.50±4.38^{a}	16.17±1.21^{bc}	2.33±0.04^{ab}
Leaves-EA	17.03±0.74^{e}	85.08±0.65^{d}	142.32±4.77^{e}	57.76±0.94^{e}	26.44±1.26^{a}	2.49±0.15^{a}
Leaves-MeOH	73.67±0.07^{d}	86.87±1.48^{d}	193.53±9.77^{d}	121.82±4.03^{d}	10.36±2.33^{d}	1.30±0.08^{d}
Leaves-Water	232.61±5.50^{b}	189.13±3.38^{b}	494.56±5.82^{b}	342.39±2.43^{b}	14.33±1.41^{c}	1.52±0.04^{d}

* Values expressed are means ± S.D. of three parallel measurements. TE: Trolox equivalent; EDTAE: EDTA equivalent. Different letters indicate significant differences in the tested extracts ($p<0.05$).

Table 4. Enzyme inhibitory properties of the ethyl acetate, methanol, and water extracts of *Ocimum americanum* flowers and leaves.

Samples	AChE inhibition (mg GALAE/g)	BChE inhibition (mg GALAE/g)	Tyrosinase inhibition (mg KAE/g)	Amylase inhibition (mmol ACAE/g)	Glucosidase inhibition (mmol ACAE/g)
Flowers-EA	4.14±0.38^{a}	3.89±0.49^{ab}	122.48±1.24^{a}	0.79±0.02^{a}	7.54±0.06^{c}
Flowers-MeOH	3.72±0.27^{a}	3.10±0.09 ^{ab}	128.33±0.09^{a}	0.78±0.02^{a}	2.25±0.46^{e}
Flowers-Water	0.89±0.07^{c}	NA	66.80±3.59^{b}	0.23±0.01^{c}	8.97±0.87^{b}
Leaves-EA	2.82±0.37^{b}	4.13±0.84^{a}	129.51±0.25^{a}	0.79±0.05^{a}	12.76±1.04^{a}
Leaves-MeOH	4.36±0.14^{a}	3.00±0.09^{b}	130.56±2.26^{a}	0.67±0.03^{b}	1.44±0.09^{e}
Leaves-Water	0.45±0.13^{c}	NA	60.22±7.52^{b}	0.15±0.01^{d}	5.91±0.33^{d}

* Values expressed are means ± S.D. of three parallel measurements. GALAE: Galantamine equivalent; KAE: Kojic acid equivalent; ACAE: Acarbose equivalent; NA: not active. Different letters indicate significant differences in the tested extracts (p<0.05).

Table 5. Minimum inhibitory concentration (MIC) of plant extracts towards selected bacterial strains.

Plant part	Extract typology (10:1)	Minimum Inhibitory Concentration (MIC) [mg (dry weight) extract mL^{-1}]*	
		S. aureus (ATCC 6538)	*S.* Typhimurium (clinical isolate)
Flowers	Ethyl acetate extract:DMSO	> 0.2	> 0.2
	Methanol extract:DMSO	0.16 (0.1-0.2)	> 0.2
	Water extract:DMSO	> 0.2	> 0.2
Leaves	Ethyl acetate:DMSO	> 0.2	> 0.2
	Methanol extract:DMSO	0.16 (0.1-0.2)	> 0.2
	Water extract:DMSO	> 0.2	> 0.2
Ciprofloxacin**	-	0.98	0.49

MIC values are reported as geometric means of three independent replicates (n=3); MIC range concentrations are reported within brackets.
** Ciprofloxacin concentration is expressed as µg mL^{-1}

Table 6. Minimum inhibitory concentration (MIC) of plant extracts towards selected yeasts and filamentous fungal strains.

		Minimum Inhibitory Concentration (MIC) [mg (dry weight) extract mL^{-1}]*	
Plant part	Extract typology (10:1)	*C. albicans* (YEPGA 6183)	*C. tropicalis* (YEPGA 6184)
Flowers	Ethyl acetate extract:DMSO	0.079 (0.05-0.1)	0.079 (0.05-0.1)
	Methanol extract:DMSO	0.031 (0.025-0.05)	0.062 (0.05-0.1)
	Water extract:DMSO	0.16 (0.1-0.2)	0.125 (0.1-0.2)
Leaves	Ethyl acetate extract:DMSO	0.079 (0.05-0.1)	0.126 (0.1-0.2)
	Methanol extract:DMSO	0.062 (0.05-0.1)	0.079 (0.05-0.1)
	Water extract:DMSO	0.125 (0.1-0.2)	>0.2
Fluconazole	-	2	4

*****MIC values are reported as geometric means of three independent replicates (n=3); MIC range concentrations are reported within brackets.

** Fluconazole concentration is expressed as µg mL^{-1}

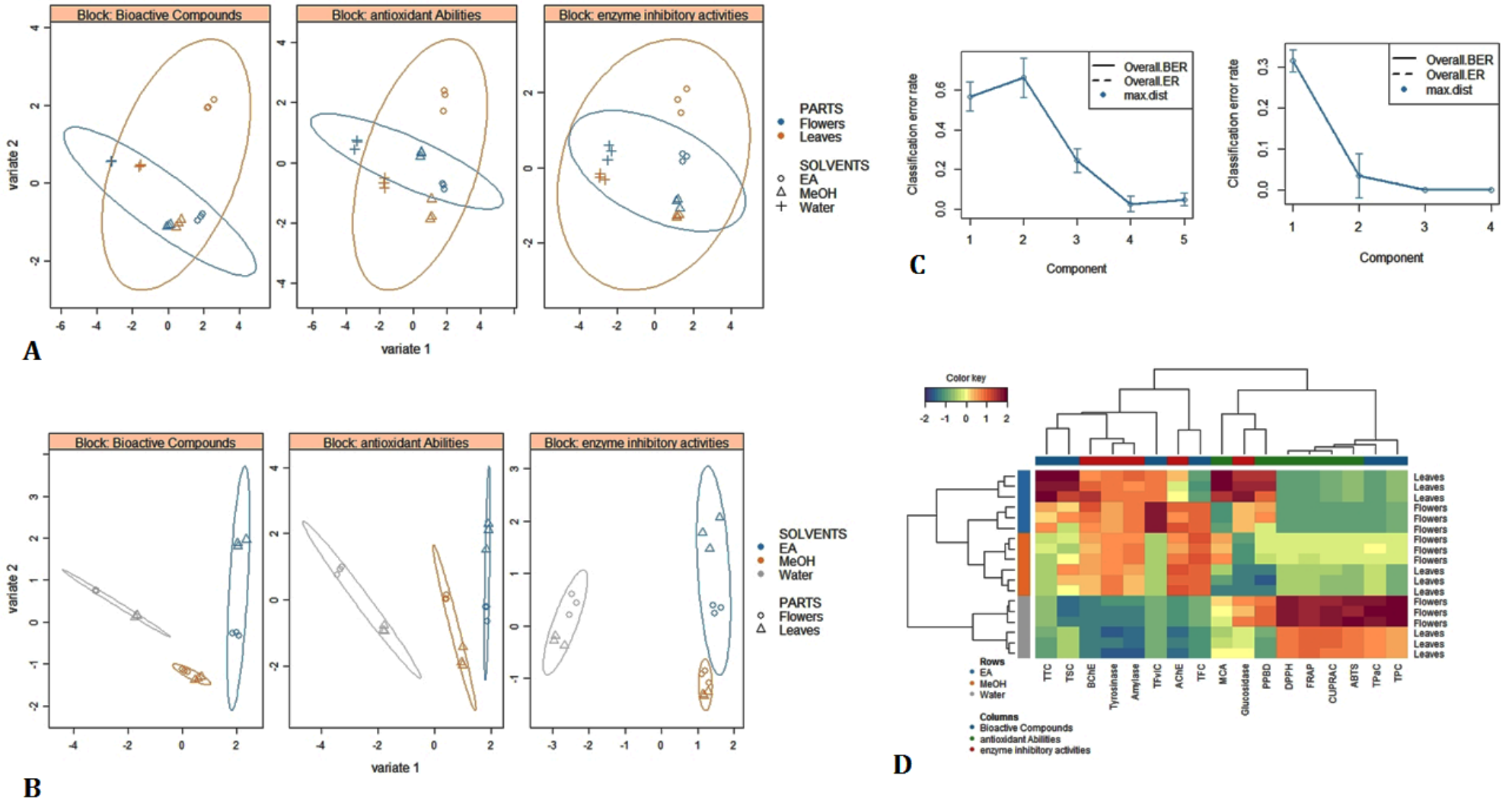
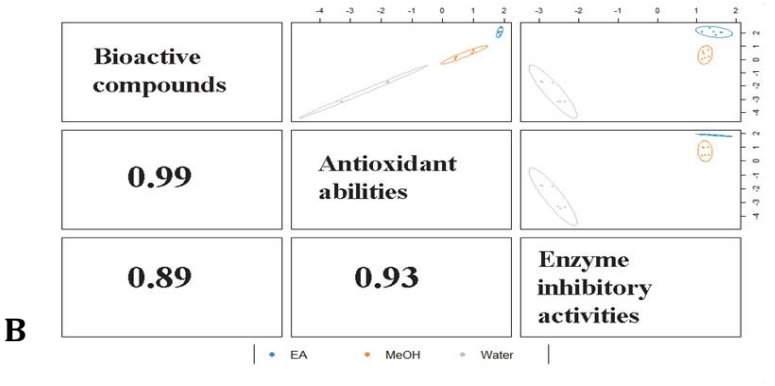

Block: Bioactive Compounds
Block: antioxidant Abilities
Block: enzyme inhibitory activities
variate 2
variate 1
PARTS
Flowers
Leaves
SOLVENTS
EA
MeOH
Water
A
Block: Bioactive Compounds
Block: antioxidant Abilities
Block: enzyme inhibitory activities
variate 2
variate 1
SOLVENTS
EA
MeOH
Water
PARTS
Flowers
Leaves
B
Classification error rate
Component
Overall.BER
Overall.ER
max.dist
C
Color key
Rows
EA
MeOH
Water
Columns
Bioactive Compounds
antioxidant Abilities
enzyme inhibitory activities
TTC
TSC
BChE
Tyrosinase
Amylase
TPvIC
AChE
TFC
MCA
Glucosidase
PPBD
DPPH
FRAP
CUPRAC
ABTS
TPaC
TPC
D

Figure 1.

	Predicted as Flowers	Predicted as Leaves
Flowers	9	0
Leaves	0	9

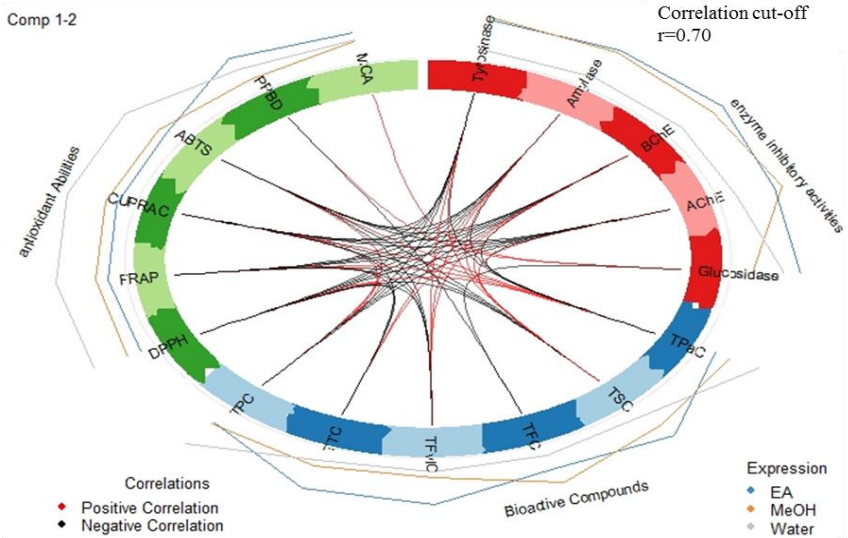
	Predicted as EA	Predicted as MeOH	Predicted as Water
EA	6	0	0
MeOH	0	6	0
Water	0	0	6

A



B

	DPPH	ABTS	CUPRAC	FRAP	PPBD	MCA
TPC	0.96	0.97	0.96	0.95	0.28	0.05
TFC	-0.50	-0.58	-0.55	-0.53	-0.41	-0.50
TFvIC	-0.74	-0.70	-0.72	-0.74	0.45	0.03
TPaC	0.99	0.98	0.98	0.98	0.02	-0.09
TTC	-0.67	-0.57	-0.63	-0.66	0.63	0.63
TSC	-0.82	-0.75	-0.79	-0.80	0.22	0.49



	AChE	BChE	Tyrosinase	Amylase	Glucosidase
TPC	-0.80	-0.84	-0.83	-0.80	0.22
TFC	0.85	0.53	0.66	0.65	-0.69
TFvIC	0.51	0.73	0.54	0.65	0.46
TPaC	-0.76	-0.91	-0.83	-0.85	-0.03
TTC	0.32	0.70	0.56	0.62	0.69
TSC	0.46	0.75	0.68	0.67	0.32

C

Figure 2.

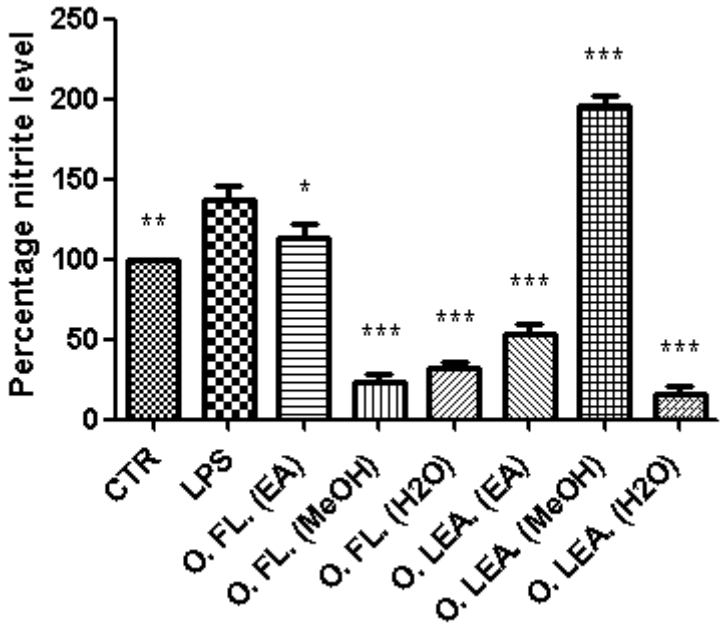


Figure 3.

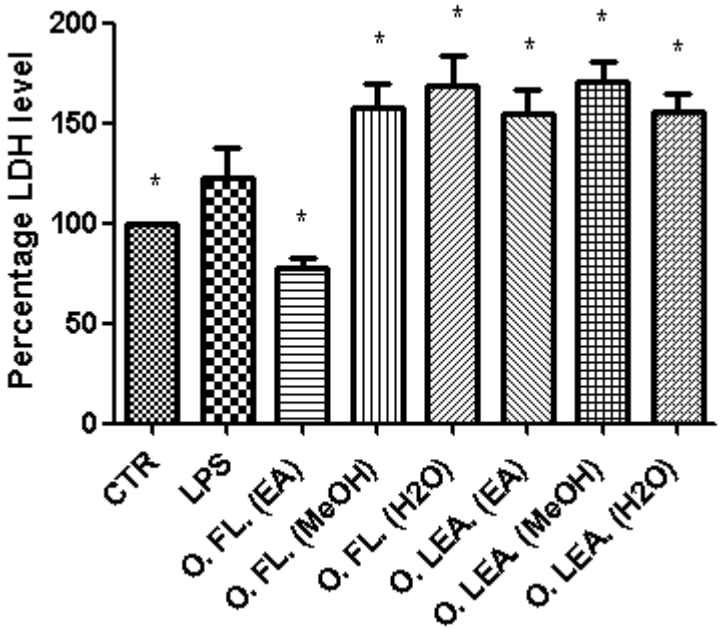


Figure 4.

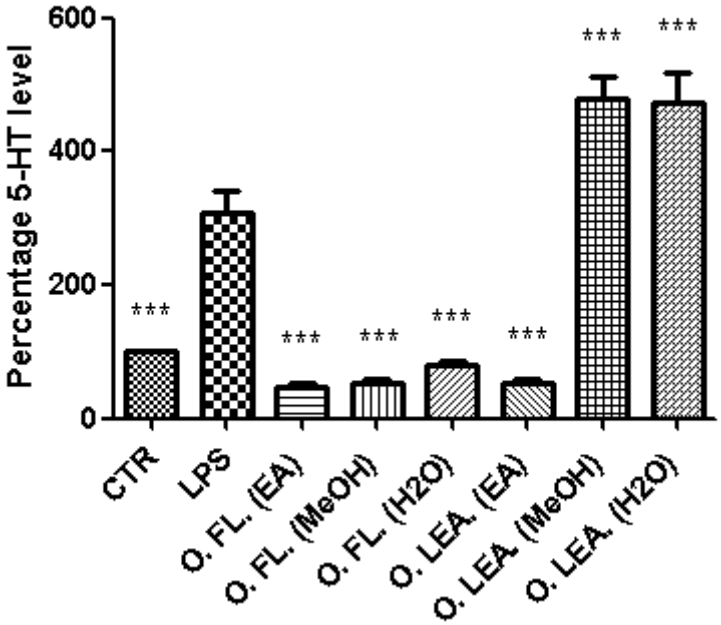


Figure 5.

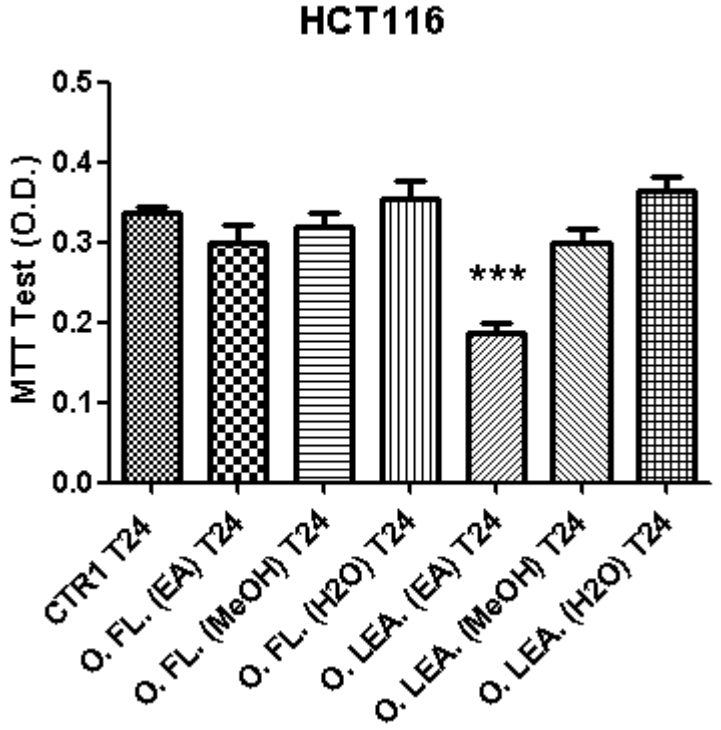


Figure 6.

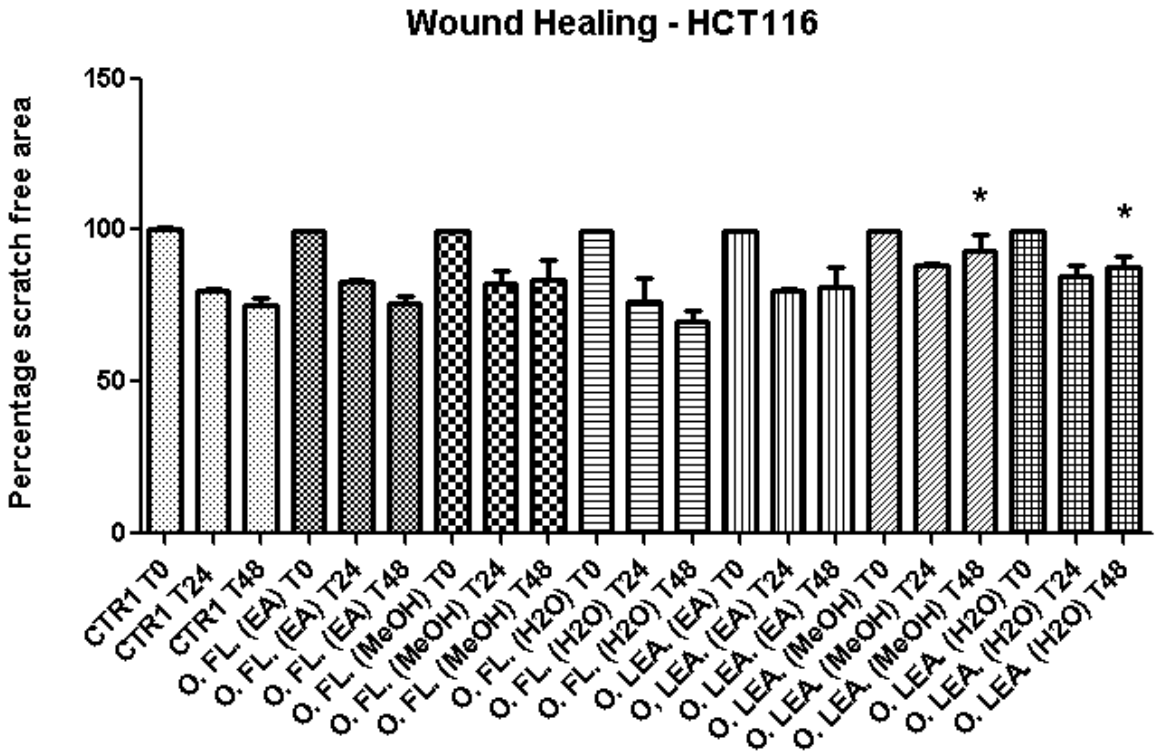

Wound Healing - HCT116
Percentage scratch free area
150
100
50
0
*
*
CTR1 T0
CTR1 T24
CTR1 T48
O. FL. (EA) T0
O. FL. (EA) T24
O. FL. (EA) T48
O. FL. (MeOH) T0
O. FL. (MeOH) T24
O. FL. (MeOH) T48
O. FL. (H2O) T0
O. FL. (H2O) T24
O. FL. (H2O) T48
O, LEA. (EA) T0
O. LEA. (EA) T24
O. LEA. (EA) T48
O. LEA. (MeOH) T0
O. LEA. (MeOH) T24
O. LEA. (MeOH) T48
O. LEA. (H2O) T0
O. LEA. (H2O) T24
O. LEA. (H2O) T48

Figure 7.