Natural Resources and Sustainable Development
Volume 11, Issue 1, 2021
DOI: 10.31924/nrsd.v11.i1.063

IN SILICO PREDICTION OF THE ANTIOXIDANT POTENTIAL AND PHARMACOKINETIC PARAMETERS OF ESSENTIAL OIL CONSTITUENTS FROM *MONODORA MYRISTICA* SEEDS

Ebhohimen E. Israel*#, Okolie P. Ngozi, Edemhanria Lawrence*, Onyijen H. Ojei*****

* Samuel Adegboyega, University, Department of Chemical Sciences, PMB 001, Ogwa, Nigeria
** University of Benin, Department of Biochemistry, PMB 1154, Benin City, Nigeria
***Samuel Adegboyega University, Department of Mathematical and Physical Sciences, PMB 001, Ogwa, Nigeria
e-mail: israel.ebhohimen@gmail.com

Abstract

Essential oils contain bioactive compounds and studies indicate diverse applications in food storage as antioxidants. Due to varying quantitative composition, it is important to identify the active ingredients and assess their individual antioxidant capacities while also considering their pharmacokinetic properties. Bioinformatics play essential role in the prediction of bioactivity as well as the toxicity of novel compounds. In this study, the antioxidant activity of the phytochemicals in Monodora myristica was predicted in silico using PASS. Systemic ADMET evaluation in the categories: physiochemical property, absorption, distribution, metabolism and excretion, of the two top-scoring compounds were analyzed using the ADMETlab free web interface. These compounds were studied alongside standard synthetic and natural antioxidants to obtain pharmacokinetic data. The parameter 'reductant' was observed as high scoring probable activity among the standard antioxidant compounds. E-beta-ocimene and carvacrol scored the highest probable activity among the compound studies. Pharmcokinetic properties of the two compounds were mostly optimal. The outcome of this in silico study provides fore knowledge to the ADMET profile of the compounds and will be useful in planning research to study their application in oxidation-induced food spoilage during cold preservation.

Key words: In silico, antioxidant, reductant, toxicity, pharmacokinetics, phytochemicals, *Monodora myristica.*

INTRODUCTION

Food preservation is an essential parameter in the food supply chain. For meat and fish as well as their products, freezing is preferred because of the capacity to retain freshness and inhibit microbial as well as enzyme action. Technological advances such as fast freezing introduced over the past decades have resulted in improved efficiency of this technique. However, freeze-induced oxidation remains a challenge (Rahman and Velez-Ruiz, 2014).

Post-mortem quality loss is high in meat and fish. Current strategies to minimize oxidation in frozen meat and fish include; removal of pro-oxidants, oxygen, or components susceptible to oxidation and the use of antioxidants (Undeland et al., 1998). Several synthetic antioxidants including butylated hydroxyl toluene (BHT), are available commercially but there is uncertainty

Corresponding author

about their toxicity (Atta et al., 2017; Edemhanria et al., 2020). Phytochemicals have been studied for diverse biological functions (Okolie et al., 2011) and have gained attention recently.

Essential oils (EOs) are aromatic oils making up only a small fraction of plant's composition. The main class of compounds that make up the EOs include; esters, aldehydes, ketones, and terpenes (Falowo et al., 2019).

Monodora myristica (*Mm*) is a perennial tree that thrives in the tropical rain forest in West Africa and all parts of the plants are useful in traditional medicine and for culinary purposes (Koudou et al., 2007). The percentage composition of essential oil in *Mm* seed is about 4.56 % (Onyenekwe et al., 1993). Available data indicate quantitative differences in phytochemical composition based on geographical location (Chalchat et al., 1997; Cimanga et al., 2002; Koudou et al., 2007). The main limitation to biochemical studies and application of crude extracts is reproducibility (Cock, 2011). This variability in quantitative composition of phytochemicals are critical factors restricting application of essential oils as antioxidants in food storage. Advances in analytical techniques now make it possible to identify individual components and even predict toxicity as well as biochemical activity.

The aim of this study was to predict the antioxidant activity and pharmacokinetic properties of the components of *Monodora myristica* essential oil as a template for further study and possible application as antioxidants in cold storage of fresh meat and fish as well as their products.

MATERIAL AND METHOD

The phytochemical composition of *Mm* was obtained from the Gas Chromatography – Mass Spectrometry (GC-MS) report by Koudou et al., 2007. The chemical structures of the compounds were downloaded from the European Bioinformatics Institute (EMBL-EBI) database with *.sdf* and *.mol* extensions. The chemical structures of natural and synthetic antioxidants; alpha tocopherol, ascorbic acid, butylated hydroxytoluene, pyrogallol and trolox (Brewer, 2011; Hamad et al., 2010; Suntato et al., 2019) were also downloaded with the same extensions and used as controls. Biological antioxidant activity was predicted using PASS web service (Lagunin et al., 2010). The *probable activity* (P_a) was set at ≥ 0.5 for the standard antioxidant compounds to select a common high scoring parameter as basis for probable antioxidant activity. The pharmacokinetic and toxicity indices of the compounds were analysed using ADMETLab web service.

The pharmacokinetic study was presented in the following categories; (a) physiochemical properties: logD pH7, LogP, LogS, (b) absorption: Caco-2 permeability, human intestinal absortion (HIA), (c) distribution: plasma protein binding, blood brain barrier (BBB), volume of distribution (VD) (d) metabolism: CYP450 1A2 inhibitor/substrate, CYP450 3A4

inhibitor/substrate, CYP450 2C19 inhibitor/substrate, CYP4502D6 inhibitor/substrate, half-life T($^1/_2$), clearance (CL) (e) toxicity: human hepatoxicity (H-HT) and Ames mutagenicity test (Dong et al., 2018). The "copy" and "combine.sdf" commands were used to combine all files with *.sdf* extension as a single file for upload to the ADMETLab web server.

RESULTS AND DISCUSSION

The capacity of phytochemicals to donate hydrogen atoms defined chemically as reduction is a key feature of effective antioxidants (Brewer, 2011). The parameter *"reductant"* was observed as the common high scoring P_a among the control compounds (> 0.60) and was set as benchmark for analyzing the constituents of the essential oil. E-beta-ocimene and carvacrol scored the highest as "*reductant*" among the test compounds at 0.562 and 0.571 respectively (Table 1).

The pharmacokinetic parameters classified as physiochemical property, absorption, distribution, metabolism, excretion and toxicity are presented in Figures 1, 2, 3 and 4 as well as Tables 2 and 3. The outcome suggests that the test compounds are lipohilic, can be absorbed, distributed metabolized and excreted.

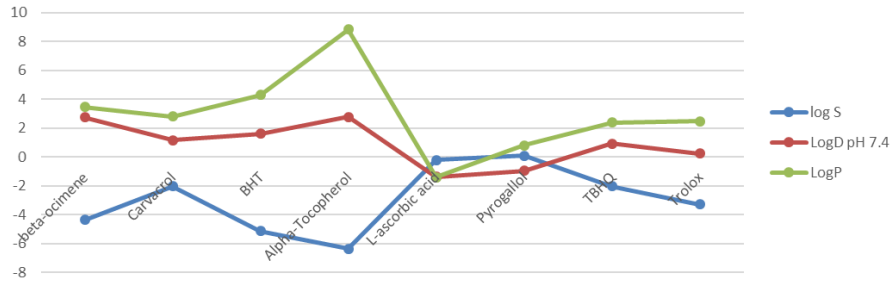

Fig 1. Predicted basic physicochemical property of phytochemicals in *Mm* seed essential oil and standard antioxidant compounds

***Ranges*:**

Log S: *<10 µg/mL= Low solubility; 10–60 µg/mL= Moderate solubility; >60 µg/mL= High solubility* (Kerns and Di, 2008, Lipinski, 2000)

LogD pH 7.4*: <1= highly soluble; 1-3= moderately soluble; 3-5=low solubility, low metabolism; >5= low solubility, high metabolism*

Log P: *<0= poor lipid bilayer permeability, 0-3= optimal, >3= poor aqueous solubility.* (Kerns and Di, 2008)

Since 1953, over five thousand five hundred and fifty nine research papers have been published on the application of EOs in foods based on their antioxidant and antimicrobial properties. Over 86 % of this number were published in the last three decades (Fernandez-Lopez and Viuda-Martos, 2018). Non-toxicity and possible application in several food systems have further increased research interest (Lucio et al., 2009). Application of

essential oils in food storage have been studied the most in fruits and vegetables (Fernadez-Lopez and Viuda-Martos, 2018).

Table 1.
Pa for Reductant Activity of Components of essential oils from *Monodora myristica* seeds

S/N	**Compound**	***Pa* (Reductant)**
1	alpha-thujene	0.266
2	alpha-pinene	0.368
3	Sabinene	-
4	beta-pinene	0.238
5	beta-myrcene	0.480
6	Alpha-phelandrene	0.333
7	p-cymene	0.398
8	Limonene	0.324
9	beta-phellandrene	-
10	E-beta-Ocimene	0.571*
11	Linalool	0.479
12	Cis-p-menth-2-enol	-
13	p-cymen-8-ol	0.461
14	Alpha-Terpineol	
15	cis-pinocarveol	-
16	Piperitol	0.400
17	Thymoquinone	0.446
18	Carvacrol	0.562*
19	alpha-santalene	0.319
20	beta-caryophyllene	0.362
21	germacrene-D	0.257
22	g-cadinene	-
23	germacrene-D-4-ol	0.298
24	caryophyllene-oxyde	-
25	T-muurolol	0.298
26	Butylated hydroxytoluene (BHT)	0.851
27	Alpha-tocopherol	0.924
28	L-ascorbic acid	0.864
29	Pyrogallol	0.604
30	tert-Butylhydroquinone (TBHQ)	0.871
31	Trolox	0.925

**Test compounds that scored the highest* P_a

Limitations to the use of crude EOs directly include; low water solubility, high volatility, strong aroma, bioavailability, varying composition and concentration (Fernadez-Lopez and Viuda-Martos, 2018). Two major approaches to overcome these limitations are controlled release by encapsulation (Asbahani et al., 2015; Fernadez-Lopez and Viuda-Martos,

2018) and incorporation into biodegradable films and coatings (Atares and Chiralt, 2016; Ribeiro-Santos et al., 2017).

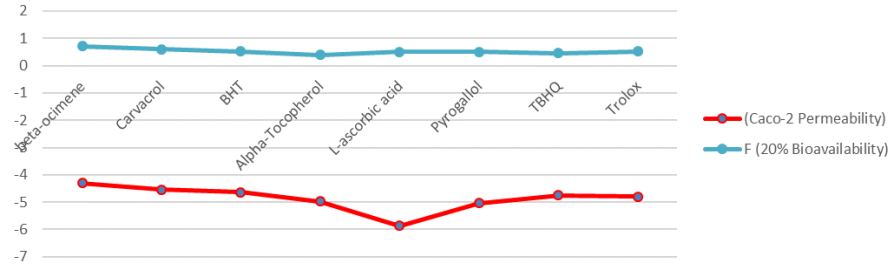

Fig 2. Predicted absorption profile of phytochemicals in *M. myristica* seed essential oil and standard antioxidant compounds.

Range:

Caco-2 Permeability: > -5.15= Optimal (Wang et al., 2016)

***HIA**: ≥30%= HIA+; <30%= HIA-* (Wang et al., 2017)

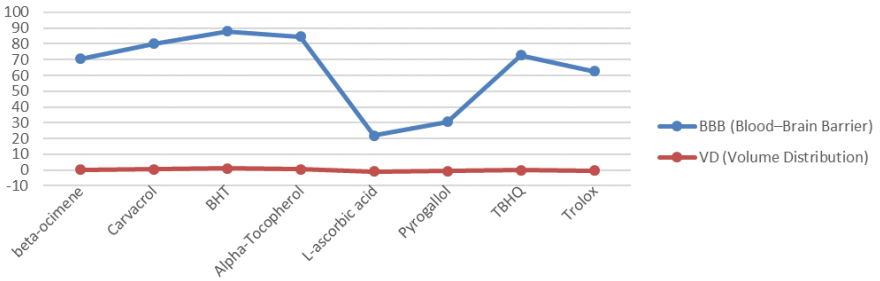

Fig 3. Predicted volume of distribution of phytochemicals in *M. myristica* seed essential oil and standard antioxidant compounds

Range:

< 0.07L/kg= confined to blood, Bound to plasma protein or highly hydrophilic;
0.07-0.7L/kg= evenly distributed;
> 0.7L/kg= bound to tissue components (e.g., protein, lipid)

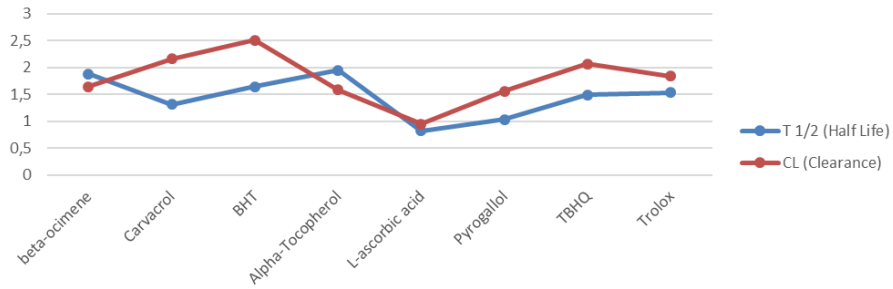

Fig 4. Predicted half-life and clearance of phytochemicals in *M. myristica* seed essential oil and standard antioxidant compounds

Range:

$T^{1}/_{2}$ (Half Life) : >8 hours= high; 3- 8 hours= moderate; <3 hours= low
CL (Clearance): >15 mL/min/kg = high; 5-15 mL/min/kg = moderate; < 5 mL/min/kg = low

Table 2

Categorization of compounds analysed as substrates/inhibitors for CYP450 enzymes.

Compounds	CYP450 1A2 inhibitor	CYP450 1A2-substrate	CYP450 3A4 inhibitor	CYP450 3A4 substrate	CYP450 2C9 inhibitor	CYP450 2C9 substrate	CYP450 2C19 inhibitor	CYP450 2C19 substrate	CYP450 2D6 inhibitor	CYP450 2D6 substrate
E-beta-Ocimene	-	-	-	-	-	+	-	-	-	+
Carvacrol	+	-	-	-	-	+	+	+	-	+
Butylated hydroxytoluene (BHT)	-	-	-	-	-	-	+	+	-	+
Alpha-tocopherol	-	-	-	+	-	+	-	-	-	-
L-ascorbic acid	-	-	-	-	-	-	-	-	-	-
Pyrogallol	-	-	-	-	-	+	-	-	-	-
tert-Butylhydroquinone (TBHQ)	-	-	-	-	-	+	-	-	-	-
Trolox	-	-	-	+	-	+	-	-	-	-

Key: + = positive; - = negative;

Table 3.

Categorization of compounds analyzed as positive/negative for toxicological processes.

Compounds	HIA (Human Intestinal Absorption)	BBB (Blood–Brain Barrier)	H-HT (Human Hepatotoxicity)	Ames (Ames Mutagenicity)
E-beta-Ocimene	+	+	-	-
Carvacrol	+	+	-	-
Butylated hydroxytoluene (BHT)	+	+	-	-
Alpha-tocopherol	+	+	+	-
L-ascorbic acid	-	+	-	-
Pyrogallol	+	+	-	+
tert-Butylhydroquinone (TBHQ)	+	+	-	-
Trolox	+	+	-	-

Key: + = positive; - = negative;

The antioxidant effect of *Aframomum angustifolium* seed essential oil in freeze stored meat was reported by (Edemhanria et al., 2020). The observed bioactivity of crude extracts may be due to the synergistic actions of several compounds, often resulting in increased bioactivities compared to individual compounds. Non-uniformity in the results from application of crude extracts is however a challenge which also affects the use of essential oils as antioxidants in foods (Cock, 2011).

In this study, the biological activities as well as the pharmacokinetic parameters of the components of *Monodora myristica* seed essential oil (Koudou *et* al., 2007) were studied *in silico* with the aim to identify compounds with antioxidant property that can be studied for use as antioxidants in food preservation (Lucio et al., 2009). The observed outcome for carvacrol obtained in this study is in agreement with the earlier reported antioxidant capacity of the compound by Brewer, 2011. Beta-ocimene is available commercially as a mixed terpene which may complicate study and possible application.

The ADMETlab webserver platform evaluates the toxicity of chemical compounds based on a comprehensive database. Toxicity is an important aspect in the scientific evaluation and application of novel compounds. The metabolic processes that xenobiotics induce are worth studying because it is relevant for designing and interpreting toxicity studies (Barton et al., 2006).

The predicted physiochemical property of chemical compounds give an insight into the solubility profile which is important for uptake, distribution and bioavailablity (Allam et al., 2011; Song et al., 2004).

Insolubility of chemical compounds limit absorption. Compounds containing ionizable groups are usually more soluble (Kern and Di, 2008).

Structural properties that affect solubility include; lipophilicity, pK_a, molecular weight and shape. The LogS (aqueous solubility) returned positive for ascorbic acid and pyrogallol due to their aqueous solubility (Sutanto et al., 2019). LogS for the other compounds were negative indicating low aqueous solubility (Fig. 1). Trolox, a water-soluble, structural analogue of alpha-tocopherol was negative possibly due to structural similarity (Figs 5 and 6) with the hydrophobic compound (Lucio et al., 2009). The outcome of *logP* prediction for test compounds was 3.47 and 2.82 respectively, indicating lipophilicity. This may enhance their antioxidant capacity in the storage of biological tissue since they can easily traverse the lipid cell membrane.

Absorption of compounds supplied orally at the intestine makes them available for metabolism and exceretion (Barton et al., 2006; Orme, 1984). The caco-2 permeability analysis for human intestinal absorption is based on the human colon epithelial cancer cell line cultured on a permeable support. In this *in silico* prediction, the test and control compounds were optimal. The human intestinal absorption (HIA) were positive based on categorization (Table 3) although the values were < 30 % (Fig 2).

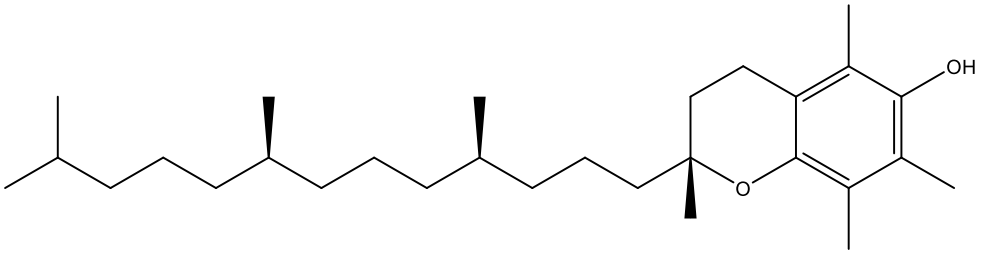

Fig 5. Alpha Tocopherol

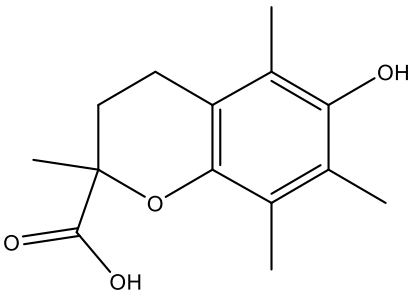

Fig 6. Trolox

The volume of distribution (VD) is a proportionality constant that relates the total amount of a compound in the body to the plasma concentration of the compound at a given time (Toutain and Bousquet-Me´lou, 2004). According to Holford and Dong-Seok, 2016, it is the second most important parameter in pharmacokinetics. The observations for VD are in line with the observed report for solubility. The scores - 0.79 L/kg, - 0.555

L/kg and – 0.40 L/kg for ascorbic acid, pyrogallol and trolox respectively indicate hydrophilicity (Fig. 3).

The two test compounds and alpha-tocopherol scored 0.38 L/kg, 0.55 L/kg and 0.44 L/kg respectively, suggesting possible even distribution (range: 0.07-0.7 L/kg). Of interest is the observed capacity for BHT (0.97 L/kg) to bind tissue components. Distribution of dietary antioxidant compounds into lipid components have been reported. Hosseini et al., 2014, reported that the dietary supplementation of BHT influenced the quality of flesh lipid of *Beluga Sturgeon* during cold storage. This may be an underlying mechanism for the reported toxicity of BHT.

The blood brain barrier (BBB) is a protective physical barrier that imposes restriction to passage of materials through the endothelial capillaries to brain. Selective permeation is essential to protect the integrity of the brain. Predictions for BBB penetration was positive for all compounds analysed (Table 3).

Upadhyay, 2014, stated that the report of throughput screening is not suitable to assess capacity to cross the BBB (de Boer et al., 2003). The concentration of additives allowed in food systems and the possible metabolic processes may make these compounds unavailable at the BBB. However, *in vivo* study to ascertain the ability to cross the BBB is suggested.

Prediction of metabolism of the selected compounds was based on their possible *activity* to act as substrates/inhibitors for/of CYP450, a superfamily of mixed function oxidases, important in the metabolism and clearance of xenobiotics. The prediction on the ADMET webserver was presented in categories as either positive (+) or negative (-). Carvacrol was predicted to be a substrate for CYP50 memebrs; 2C9, 2C19 and 2D6. It was also predicted not to inhibit 3A4, 2C9 and 2D6. The overall prediction suggests susceptibility to *in vivo* metabolic processes that will favour clearance. Comparatively, predictions for carvacrol were more favourable compared to BHT.

Toxicity was predicted as human hepatotoxicity (H-HT) and Ames mutagenicity test. The test compounds were categorized negative. In pharmacokinetics, the half-life (T1/2) of a compound is inversely proportional to the rate of clearance. The predictions for T1/2 for all compounds was < 3 hours suggesting a short half-life. The predicted clearance was also low (< 5mL/min/Kg) although it is expected to be high considering the inverse relationship between the two parameters.

CONCLUSIONS

Prediction of bioactivity and pharmacokinetic property provide background information that can guide research study on the toxicity and

application phytochemicals. Although i*n silico* methods are not final determinants of the ADMET profile for chemical compounds, the information gleaned from this research will guide the research on toxicity and antioxidant capacity of carvacrol as a potential substitute for synthetic antioxidants to delay oxidation during cold storage of lipid rich tissues.

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Received: October 13, 2020
Revised: November 17, 2020
Accepted and published online: February 28, 2021