Current Trends in Biotechnology and Pharmacy
Vol. 16 (Supplemetry Issue 1) 56 - 63, 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2022.2s.31

Synergistic Effect of *Ocimum sanctum* and *Piper nigrum*: An *In Vitro* Study on Type 2 Diabetes-Related Enzymes and MCF-7 Breast Cancer Cell line

Jayasindu Mathiyazhagan[1], Harishkumar Madhyastha[2], E Nalini[1] and K. M. Gothandam[1]*

[1]School of Bio Sciences and Technology, Vellore Institute of Technology, Vellore-14, Tamil Nadu, India
[2]Department of Applied Physiology, Faculty of Medicine, University of Miyazaki, Miyazaki 889-1692, Japan
*Corresponding Author :gothandam@gmail.com

Abstract

Ocimum sanctum and *Piper nigrum* are medicinal plants traditionally used in Ayurveda and other ancient therapeutic systems. Till today people use these plants in one of the different aspects of their everyday life. This study revealed the combined effect of *O. sanctum* and *P. nigrum* (1:1) against diabetic-related enzymes in a cell-free system and breast cancer cell line (MCF-7). The HPLC analysis revealed that Eugenol and Piperine are the major phytocompounds in *O. sanctum* and *P. nigrum,* respectively. Digestive enzymes like α- amylase and α- glucosidase assays were carried out to find their antidiabetic effect. To determine the interaction between Piperine and eugenol with enzymes involved in diabetes, the *in silico* molecular docking analysis was performed. The plant extracts alone and in combination, were checked for their cytotoxicity and reactive oxygen species levels in MCF-7 cells.

Keywords: *Ocimum sanctum*; *Piper nigrum*; HPLC; antidiabetic; Docking analysis; anticancer.

Introduction

Diabetes is a metabolic disorder caused due to an imbalance in the body's blood glucose level. Type 2 diabetes is commonly diagnosed in people between the age of 20-79. The primary symptom of type 2 diabetes is hyperglycemia (increased blood sugar level). Mostly dysfunction of functional pancreatic cells and inadequate secretion of glucagon leads to type 2 diabetes. The likelihood of type 2 diabetes patients acquiring cardiovascular disease is very high [1, 2]. WHO (World Health Organization) report states that 171 million populations suffered from diabetes in 40 years. In 2013, individuals with diabetes were increased to 382 billion [3]. International Diabetes Federation (IDF) determines, by 2035, the rate of diabetes patients might increase to 591.5 million [4].

Breast cancer is a significant threat among women around the world. According to WHO, in India, breast cancer accounts for 14% cancer incidence and 11.1% mortality rates. There are three significant categories of breast cancer based on estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor 2 (HER2): (i) ER/PR +ve /HER2 -ve, (ii) HER2 +ve, and (iii) triple-negative. The primary treatments are chemotherapy, radiotherapy, and surgery. For centuries, herbal plants were used for the prevention of various diseases. Two such plants are the *Ocimum sanctum* and *Piper nigrum*.

Ocimum sanctum is one of the medicinal herbs which belong to the family *Lamiaceae*. It is commonly known as Tulsi or Holy Basil in English. It is the holistic herb that is worshipped

Vol. 16 (Supplemetry Issue 1) 56 - 63, 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2022.2s.31

by Hindus. Different plant parts of tulsi have various medicinal properties. Hence roots, leaves, and seeds were traditionally used in ayurvedic and other ancient medicines [5]. *O. sanctum* has various medicinal properties including analgesic, anticancer activity, anti-inflammatory activity, antidiabetic activity, antioxidant, anti-stress, anti-ulcer activity, anti-fertility agent. It is also used to treat arthritis, heart diseases, malaria, and skin diseases [6, 7]. The primary phenolic component present in the *O. sanctum* plant is eugenol, one of the bioactive compounds with therapeutic and pharmaceutical activity [8]. Eugenol can also act as a biomarker in some cases and is utilized in the food industry and as a flavoring agent [9]. *Piper nigrum*, commonly known as 'Pepper,' is another medicinally important plant belonging to the *Piperaceae* family. Generally, it is considered the "king of spices" as it is frequently used as spices in many dishes. It has many medicinal properties, including anti-tumor activity, anti-depressant, anti-diarrhea, and analgesic activities [10]. *P. nigrum* is abundant with glutathione peroxidase and glucose 6 phosphate dehydrogenase enzymes [11]. Piperine is one of the alkaloids present in *P. nigrum*. Piperine is the bioactive agent found in black pepper. Piperine is responsible for the antioxidant property of pepper [12]. It is used to treat cough, bronchitis, asthma, etc. [13].

In the present study, *O. sanctum,* and *P. nigrum* alone and in combination were tested against enzymes (α-amylase and α-glucosidase) related to antidiabetic activity. and cytotoxic activity in breast cancer cell lines (MCF-7).

Materials and Methods

***Preparation of extracts and characterization*:** The plant extracts (12.5g) were taken in 200 ml distilled water and decocted till it was reduced to four folds (50ml) [14]. The decoction was centrifuged at 2000 rpm for five minutes. The remaining supernatant was collected in the tubes and preserved at -80°C. The extracts were freeze-dried and used for further analysis.

Quantitative estimation of eugenol and Piperine present in *O. sanctum* and *P. nigrum,* respectively, was performed using HPLC. The analysis was conducted in Shimadzu modular HPLC (Kyoto, Japan, Model: CBM-20A) coupled with a diode array detector (SPD-20A) and pumps (LC-20AT) linked to an injection valve (20 µl volume). The system is incorporated of C_{18} reverse-phase column (Phenomenex Luna, 250X4.6 mm, 5 µm). For eugenol, the mobile phase used is the solution containing Methanol, Acetonitrile, and Water (10:50:40), respectively. Isocratic solvents are employed for this purpose, and the wavelength chosen for *O. sanctum* is 280 nm [15]. The mobile phase for Piperine is Acetonitrile, Water, and Acetic acid (60:39.5:0.5), which was employed for *P. nigrum* at 340 nm wavelength [16]. 1mg/ml is the concentration used for both standards as well as extracts.

In vitro antidiabetic assays in cell-free systems

Alpha-amylase assay: Different concentrations of the standard drug acarbose and plant extracts alone and in combination (1:1) (10, 50, 100, 150, 200 µg/ml) were prepared by methanol. From each tube, 10µl of standard and extracts were aliquoted into the 96 wells microplate reader. To each well-containing extract, 10µl of α- amylase enzyme was added. The enzyme was diluted in sodium phosphate buffer (0.02M) and incubated at 25°C for 10 minutes. Then 1% of starch was introduced to the plate and again kept at 25ºC for 10 minutes. Further to the solution 20µl DNSA was added and kept for 5 min at 90ºC. 200µl of distilled water is added after incubation. The absorbance was taken at 540 nm using microplate reader analysis [17]. The activity of α- amylase inhibition is calculated by using the formula.

$$\% \text{ Inhibition of } \alpha\text{-amylase} = \frac{(\text{Absorbance of control- Absorbance of sample})}{(\text{Absorbance of control})} \times 100$$

***Alpha-glucosidase assay*:** Various concentrations of acarbose and plant extracts (10, 50, 100, 150, 200 µg/ml) were made using methanol. For

Vol. 16 (Supplemetry Issue 1) 56 - 63, 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2022.2s.31

every 50µl of the extract and standard solution, 100µl of α - glucosidase was added. The microplate was kept for incubation at 25ºC for 10 minutes. After adding 50µl of P-nitrophenyl-α-D-glucopyranoside, the plate was again incubated at 25ºC for 5 minutes. Microplate reader analysis was carried out for measuring absorbance at 405 nm [17]. The activity of α-glucosidase inhibition is calculated by using the formula mentioned below.

$$\% \text{ Inhibition of } \alpha\text{-glucosidase} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{(\text{Absorbance of control})} \times 100$$

***In vitro* anti-breast cancer analysis:**

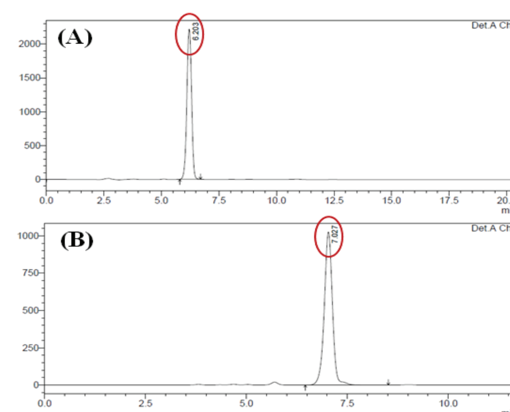
Cytotoxicity assay: The MCF-7 cell line was procured from NCCS, Pune. The cytotoxicity of extracts to the MCF-7 cells were measured by MTT (3-(4,5-dimethylthiazol-2-yl)2,5diphenyltetrazolium bromide) assay. The cells were kept in 5% CO_2 at 37°C incubator. The MCF-7 cells were incubated with different concentrations (10, 50, 100, and 200 µg/mL) of *O. sanctum* and *P. nigrum* alone and in combination (1:1) for 24 h. Addition of 20 µL of MTT at 2.5 mg/mL concentration was introduced to the wells after 24 hours of treatment. MTT was removed after 4 hours from the wells, and the DMSO was used to dissolve the formazan crystals to measure metabolically active cells at 570 nm wavelength. Cell morphology after treatment was observed using an inverted phase-contrast microscope.

Molecular docking analysis: The required protein α-amylase (5U3A) and α-glucosidase (5NN3) were retrieved from the Protein Databank (PDB). Then the heteroatoms were removed, and the file was saved. The ligands such as Piperine and eugenol were taken from the PubChem database. Autodock 4 was used for docking simulation to run and to find the interaction among protein and ligand. The better confirmation of ligand was selected based on the lowest binding energy and viewed by pymol. Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) of eugenol and Piperine were predicted by pkCSM software [18].

Determination of ros using flow cytometry: The levels of ROS were evaluated by the 2',7'-dichlorofluorescein diacetate (DCFH-DA) method using flow cytometry. The IC50 concentrations of *O. sanctum* and *P. nigrum* alone and in combination (1:1) were added to MCF-7 cells. The cells were trypsinized after 24 hours and incubated with 20 µM DCFH-DA in darkness for 30 minutes at 37 ˚C. The ROS levels were measured using flow cytometry, and the results were analyzed using the software cytexpert (CytoFLEX, Beckman Coulter, USA).

Results and Discussion

Characterization of O. sanctum and P. nigrum by HPLC analysis: The presence of the primary therapeutic constituents eugenol (4 µg/mg) in *O. sanctum* and Piperine (267 µg/mg) in *P. nigrum* were carried out using HPLC analysis (Figure 1). Eugenol was reported to prevent type 2 diabetes by enhancing glucose uptake in skeletal muscle and increased insulin sensitivity in rat models [19]. Eugenol exhibited anticancer activity in breast cancer cell lines and mice models [20]. It was also reported to induce apoptosis in the MCF-7 cell line [21] and anti-metastatic activity in MDA-MB-231 (triple-negative) and SK-BR-3 (HER2 positive) cell lines [22]. Piperine exhibited antidiabetic activity by reducing blood glucose levels and increasing insulin sensitivity [23]. Piperine also showed anti-proliferative effect in MDA-MB-231 cell lines [24].



Current Trends in Biotechnology and Pharmacy
Vol. 16 (Supplemetry Issue 1) 56 - 63, 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2022.2s.31

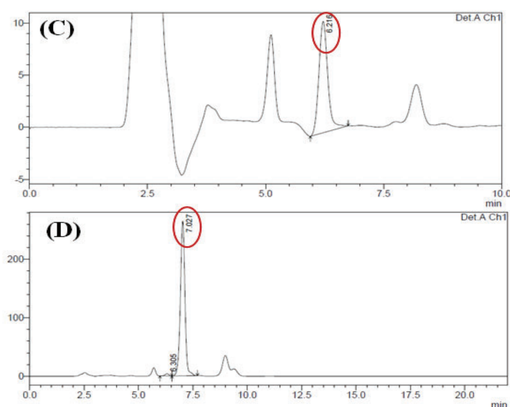


Figure 1: HPLC analysis of (A) Standard Eugenol, (B) Standard Piperine, (C) Eugenol in *O. sanctum*, and (D) Piperine in *P. nigrum*.

Antidiabetic activities in the cell-free system: The α-amylase and α-glucosidase are the major enzymes present in the pancreas and small intestine, respectively. These enzymes role is to break down insoluble starch into simple compounds, which increases the blood glucose levels. Certain medicinal plants can produce α-amylase and α-glucosidase inhibitors that are accountable for inhibiting the activity of these enzymes. Therefore plays a crucial role in curing diabetes [25]. In some cases, α amylase can be administered as an oral drug to treat type 2 diabetes [26]. *O. sanctum* can lower the sugar level in diabetes patients [27]. Piperine also has the hypoglycemic property. Hence, *P. nigrum* can minimize the glucose level in the blood [28].

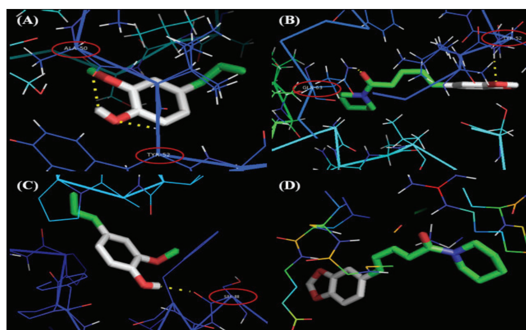


Figure 2: (A) Inhibition of α-amylase enzyme activity, and (B) Inhibition of α-glucosidase enzyme activity.

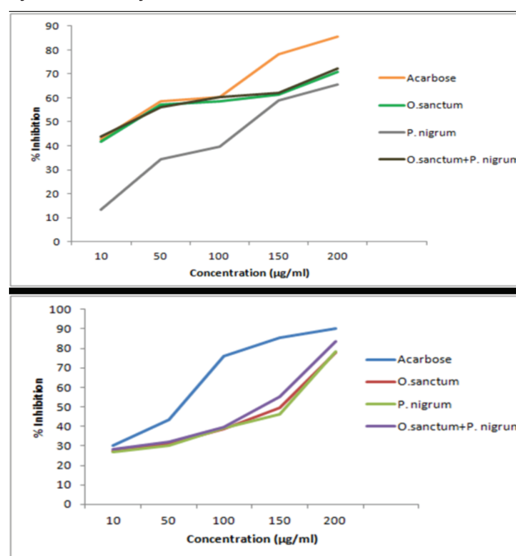


Figure 3: Visualization of docked complexes by Pymol. (A) Eugenol docked with α-amylase (B) Piperine docked with α-amylase (C) Eugenol docked with α-glucosidase, and (D) Piperine docked with α-glucosidase.

Table 1: Canonical smiles, molecular weight, and ADMET properties of Eugenol and Piperine

Ligands	Eugenol	Piperine
Canonical Smiles	COC1=C(C=C-C(=C1)CC=C)O	C1CCN(CC1)C(=O)C=C-C=CC2=C-C3=C(C=C2)OCO3
Mol. Weight (g/mol)	164.2	285.34
Absorption-Intestinal absorption (human) %	92.014	94.444
Distribution BBB Permeability (log BB)	0.374	-0.102

Vol. 16 (Supplemetry Issue 1) 56 - 63, 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2022.2s.31

Metabolism	CYP1A2 inhibitor	CYP3A4 and CYP2C19 inhibitor
Excretion- Total clearance (log ml/min/kg)	0.282	0.232
Toxicity-AMES/Hepatotoxicity	Yes/No	No/Yes

Table 2: Docking scores and molecular interactions between proteins and ligands.

Protein-ligand	α-amy: Eug	α-amy: Pip	α-glu: Eug	α-glu: Pip
Binding Energy (kcal/mol)	-4.06	-5.89	-4.87	-5.44
No. of H-bond formed	2	2	1	0
Active site involved in H-bonding	ALA-50 and TYR-52	GLN-63 and TYR-52	SER-88	-

(α-amy: α-amylase | α-glu: α glucosidase | Eug: Eugenol | Pip: Piperine)

O. sanctum and P. nigrum alone and in combination were evaluated for their *in vitro* α-amylase and α-glucosidase enzyme inhibitory studies (Figure 2A-2B). The standard drug acarbose exhibited the highest inhibitory activities on both enzymes. The concentration-dependent inhibition against enzymes was observed in all the extracts. The IC50 values of acarbose, *O. sanctum*, *P. nigrum,* and *O. sanctum* + *P. nigrum* to inhibit α-amylase enzyme was presented to be 32.41 µg/mL, 40.77 µg/mL, 130.60 µg/mL and 32.32 µg/mL respectively. The IC50 of acarbose, *O. sanctum*, *P. nigrum,* and *O. sanctum* + *P. nigrum* to inhibit α-glucosidase enzyme was presented to be 57.3 µg/mL, 122 µg/mL, 125.18 µg/mL, and 109.70 µg/mL, respectively. The inhibition of both the enzymes was noted to be high in combination when compared to individual extracts. It showed the synergistic activity of *O. sanctum* and *P. nigrum*.

The *in silico* molecular interaction between major phytoconstituents of *O. sanctum* (eugenol) and *P. nigrum* (Piperine) with α-amylase and α-glucosidase enzymes showed high binding energies (Table 2). The bioavailability and ADMET properties of eugenol and Piperine were noted (Table 1). The molecular interactions and the hydrogen bonds were viewed using pymol (Figure 3).

Cytotoxicity and apoptosis in MCF-7 cell line: The combination of *O. sanctum* and *P. nigrum* is reported for the first time in our study. The cytotoxicity of *O. sanctum* and *P. nigrum* alone and in combination (1:1) with various concentrations in the MCF-7 cell line was estimated (Figure 4). As seen, all the extracts showed dose-dependent cytotoxicity, whereas *O. sanctum* + *P. nigrum* showed high cytotoxicity than individual extracts. The IC50 values of *O. sanctum*, *P. nigrum* and *O. sanctum* + *P. nigrum* was presented to be 206.83 µg/mL, 109.27 µg/mL, and 78.61 µg/mL respectively. The obtained data showed the combination has a synergistic effect in reducing the proliferation of breast cancer cells. Incubation of *O. sanctum* and *P. nigrum* alone and in combination (1:1) with IC50 concentrations showed increased cell death, chromatin condensation, and shrunken cytoplasm observed in phase-contrast microscopy (Figure 5). Additionally, the combined extract showed apoptotic bodies. The treatments were compared with control cells, which showed intact morphology with a normal polygonal shape.

The intracellular ROS levels were measured using flow cytometry to observe apoptosis (Figure 6). The increased ROS levels were observed with the increased intensity of fluorescence. Control cells showed 22.87% ROS levels, whereas *O. sanctum*, *P. nigrum,* and *O. sanc-*

Vol. 16 (Supplemetry Issue 1) 56 - 63, 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2022.2s.31

tum + *P. nigrum* showed 38.47%, 32.36% and 49.07% ROS levels, respectively. As observed, *O. sanctum* + *P. nigrum* showed high ROS levels than individual extracts. The results showed the combined *O. sanctum* + *P. nigrum* has high cytotoxicity and apoptosis in MCF-7 cell line. There are previous reports on *O. sanctum* and *P. nigrum* individually against type 2 diabetes and breast cancer. This study is the first report on the combined effect of *O. sanctum* and *P. nigrum* against type 2 diabetes and breast cancer. Additional studies are required to state the mechanism of action clearly.

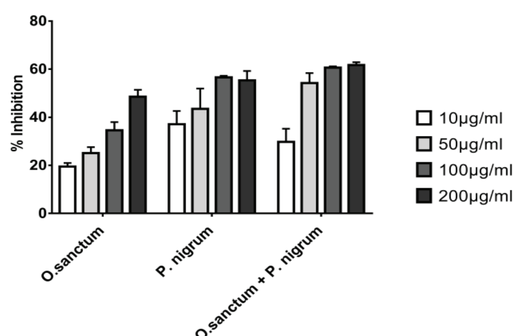


Figure 4: Cytotoxicity analysis of *O. sanctum*, *P. nigrum* and *O. sanctum* +*P. nigrum* in MCF-7 cell line.

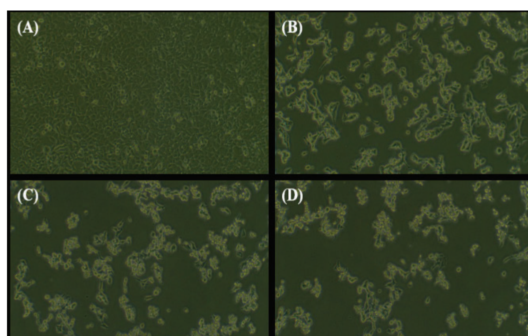


Figure 5: Morphological analysis using phase-contrast microscope in MCF-7 cell line. (A) Control cells without treatment, (B) Cells treated with IC50 concentration of *O. sanctum* (C) Cells treated with IC50 concentration of *P. nigrum*, and (D) Cells treated with IC50 concentration of *O. sanctum* +*P. nigrum*.

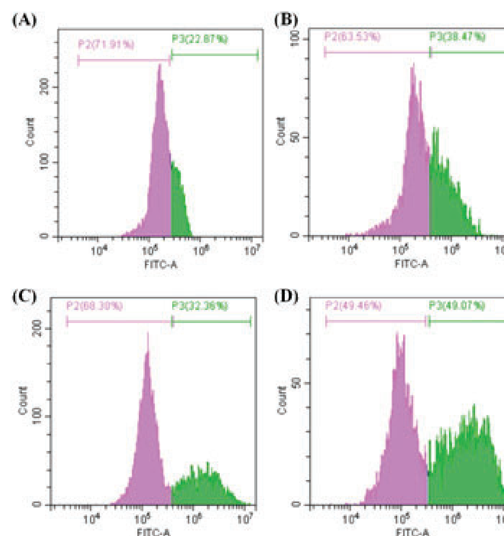


Figure 6: Flow cytometry analysis to measure intracellular ROS levels. (A) Control cells without treatment, (B) Cells treated with IC50 concentration of *O. sanctum* (C) Cells treated with IC50 concentration of *P. nigrum*, and (D) Cells treated with IC50 concentration of *O. sanctum* +*P. nigrum*.

Ethics Statements

Not applicable.

Conflict of interest

The author declares no conflict of interest.

Acknowledgement

The authors would like to thank the Vellore Institute of Technology for the facilities and financial support.

References

1. Sposito, A.C., Berwanger, O., de Carvalho, L.S.F., and Saraiva, J.F.K. (2018) Glp-1ras in Type 2 Diabetes: Mechanisms that Underlie Cardiovascular Effects and Overview of Cardiovascular Outcome Data. Cardiovas diabetol. 17 (1): 1-19.
2. Dash, S., Pattnaik, G., Kar, B., Sahoo, N.,

Vol. 16 (Supplemetry Issue 1) 56 - 63, 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2022.2s.31

& Bhattacharya, S. (2021) An Approach Towards Method Development to Investigate the Anti-Diabetic Activity on Experimental Animals. Curr. Trends Biotechnol. Pharm., *15*(3): 330–348.

3. Ogurtsova, K., da Rocha Fernandes, J., Huang, Y. et al. (2017) IDF Diabetes Atlas: Global Estimates for the Prevalence of Diabetes for 2015 and 2040. Diabetes Res Clin Pract. 128: 40-50.

4. Beagley, J., Guariguata, L., Weil, C., and Motala, A.A. (2014) Global Estimates of Undiagnosed Diabetes in Adults. Diabetes Res Clin Pract. 103 (2): 150-160.

5. Pandey, G. and Madhuri, S. (2010) Pharmacological Activities of *Ocimum sanctum* (Tulsi): A Review. Int. J Pharm Sci Rev Res. 5 (1): 61-66.

6. Mehta, V., Sharma, A., Kailkhura, P., and Malairaman, U. (2016) Antioxidant, Anti-Inflammatory, and Antidiabetic Activity of Hydroalcoholic Extract of *Ocimum sanctum*: An in-vitro and in-silico study. Asian J Pharm Clin Res. 9 (5): 44-49.

7. Faujdar, C., & Priyadarshini. (2021) Evaluation of In-vitro Cytoprotective, Wound Healing and Antioxidant Effects of *Ocimum sanctum* Leaf Extract Chetna. Curr. Trends Biotechnol. Pharm., *15*(3), 248–255.

8. Khalil, A.A., ur Rahman, U., Khan, M.R. et al. (2017) Essential Oil Eugenol: Sources, Extraction Techniques and Nutraceutical Perspectives. RSC Adv. 7 (52): 32669-32681.

9. Joshi, A., Sharma, V., and Dubey, B. (2011) Development of an Analytical Method to Determine Eugenol Concentrations in Alcoholic Extracts of Different Species of Ocimum's. Int J Phytopharm Res. 1: 35-42.

10. Ahmad, A., Husain, A., Mujeeb, M., Khan, S.A., Alhadrami, H.A.A., and Bhandari, A. (2015) Quantification of Total Phenol, Flavonoid Content and Pharmacognostical Evaluation Including HPTLC Fingerprinting for the Standardization of Piper Nigrum Linn Fruits. Asian Pac. J. Trop. Biomed. 5 (2): 101-107.

11. Abou-Elkhair, R., Ahmed, H., and Selim, S. (2014) Effects of Black Pepper (*Piper nigrum*), Turmeric Powder (*Curcuma longa*) and Coriander Seeds (*Coriandrum sativum*) and Their Combinations as Feed Additives on Growth Performance, Carcass Traits, Some Blood Parameters and Humoral Immune Response of Broiler Chickens. Asian Austral J Anim. 27 (6): 847.

12. Srinivasan, K., (2013) Biological Activities of Pepper Alkaloids', in Ramawat K., M.J. (ed.). Natural Products. Springer-Verlag Berlin Heidelberg.

13. Ganesh, P., Kumar, R.S., and Saranraj, P. (2014) Phytochemical Analysis and Antibacterial Activity of Pepper (*Piper nigrum* L.) against Some Human Pathogens. Cent Eur J Exp Biol. 3 (2): 36-41.

14. Thakkur CG (1974) Introduction to Ayurveda: The science of life. Red Wheel/Weiser.

15. Inam, F., Deo, S., and Narkhede, N. (2014) HPLC–UV method Development and Quantification of Eugenol from Methanolic Extracts of Some Spices. Int J Chem Phys. 3 (6): 92-102.

16. Upadhyay, V., Sharma, N., Joshi, H.M., et al. (2013) Development and Validation of Rapid Rp-HPLC Method for Estimation of Piperine in *Piper nigrum* L. Int J Herb Med. 1 (4): 6-9.

17. Oboh, G., Akinyemi, A., and Ademiluyi, A. (2012) Inhibition of A-Amylase and A-Glucosidase Activities by Ethanolic Extract of Telfairia Occidentalis (Fluted Pumpkin) Leaf. Asian Pac J Trop Biomed. 2 (9): 733-738.

18. Pires, D.E., Blundell, T.L., and Ascher, D.B.

Vol. 16 (Supplemetry Issue 1) 56 - 63, 2022, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2022.2s.31

(2015) PKCSM: Predicting Small-Molecule Pharmacokinetic and Toxicity Properties Using Graph-Based Signatures. J Med Chem. 58 (9): 4066-4072.

19. Al-Trad, B., Alkhateeb, H., Alsmadi, W. and Al-Zoubi, M. (2019) Eugenol Ameliorates Insulin Resistance, Oxidative Stress and Inflammation in High Fat-Diet/Streptozotocin-Induced Diabetic Rat. Life Sci. 216: 183-188.

20. Al-Sharif, I., Remmal, A., and Aboussekhra, A. (2013) Eugenol Triggers Apoptosis in Breast Cancer Cells through E2f1/Survivin Down-Regulation. BMC Cancer. 13 (1): 1-10.

21. Vidhya, N. and Devaraj, S.N. (2011) Induction of Apoptosis by Eugenol in Human Breast Cancer Cells. Indian Journal of Experimental Biology. 49 (11): 871-878.

22. Abdullah, M.L., Hafez, M.M., Al-Hoshani, A., and Al-Shabanah, O. (2018) Anti-Metastatic and Anti-Proliferative Activity of Eugenol against Triple Negative and Her2 Positive Breast Cancer Cells. BMC Compl. Alternative Med. 18 (1): 1-11.

23. Shreya, S., Gaurang, S., Mandev, P., and Satbeer, S. (2010) Effect of Piperine in Obesity Induced Insulin Resistance and Type-Ii Diabetes Mellitus in Rats. J Nat Remedies. 10 (2): 116-122.

24. Greenshields, A.L., Doucette, C.D., Sutton, K.M. et al. (2015) Piperine Inhibits the Growth and Motility of Triple-Negative Breast Cancer Cells. Cancer Lett. 357 (1): 129-140.

25. Kazeem, M., Adamson, J., and Ogunwande, I. (2013) Modes of Inhibition of A-Amylase and A-Glucosidase by Aqueous Extract of *Morinda lucida* Benth Leaf. BioMed Res Int. 2013

26. Nair, S.S., Kavrekar, V., and Mishra, A. (2013) In Vitro Studies on Alpha Amylase and Alpha Glucosidase Inhibitory Activities of Selected Plant Extracts. Eur J Exp Biol. 3 (1): 128-132.

27. Mehta, V., Sharma, A., Kailkhura, P., and Malairaman, U. (2016) Antioxidant, Anti-Inflammatory, and Antidiabetic Activity of Hydroalcoholic Extract of *Ocimum sanctum*: An in-Vitro and in-Silico Study. Asian J Pharm Clin Res. 9 (5): 44-49.

28. Khaliq, T., Sarfraz, M., and Ashraf, M. (2015) Recent Progress for the Utilization of Curcuma longa, *Piper nigrum* and *Phoenix dactylifera* Seeds against Type 2 Diabetes. West Indian Med J. 64 (5): 527.