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Role of the Phytochemicals from the Cocoa Shell on the Prevention of Metabolic Syndrome by an Integrated Network Pharmacology Analysis †

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Abstract: The metabolic syndrome is a cluster of conditions that occur together, increasing the risk of chronic diseases such as obesity, type II diabetes, or cardiovascular disease. Nutritional interventions improving inflammation, bioenergetics, and oxidative stress are proposed as effective tools in preventing metabolic syndrome. The cocoa shell is a by-product generated in large amounts during cocoa production. This by-product contains alkaloids and phenolic compounds as main compounds and has been investigated as an anti-obesity and anti-diabetic agent. Here, we used network pharmacology to explore the potential mechanism of the phytochemicals from the cocoa shell. We searched the cocoa shell's phytochemical composition, and oral bioavailability and drug-likeness were screened. We predicted the cocoa shell phytochemicals' targets and used different databases to search for compound-protein and compound-gene interactions, and then protein-protein interaction networks were constructed. Moreover, pathway enrichment analysis was performed, and biological processes and signaling pathways were identified and illustrated through bioinformatics analyses. Theobromine, caffeine, procyanidin B2, (−)-epicatechin, (+)-catechin, and protocatechuic acid were identified in the cocoa shell as main components. Those phytochemicals were associated with inflammation- (TNF-α, NF-κB, JNK), oxidative stress (CAT, SOD), obesity- (adiponectin, leptin, FASN, PPAR-α), and diabetes- (insulin, AKT,) related pathways. Results demonstrated that cocoa shell phytochemicals could modulate multiple biological processes and signaling pathways in a multimechanistic manner. Hence, cocoa shell consumption could support the nutritional prevention of metabolic syndrome. Future *in vivo* and clinical investigations will be needed to validate this potential nutraceutical and healthy ingredient's effects and mechanisms.

Keywords: metabolic syndrome; obesity; diabetes; cardiovascular disease; cardiometabolic disease; inflammation; oxidative stress; cocoa shell; cocoa by-products; phytochemicals; theobromine; caffeine; phenolic compounds; network pharmacology; bioinformatic analysis; signaling pathway

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1. Introduction

Metabolic syndrome is a group of clinical conditions and disorders, including obesity, insulin resistance, glucose intolerance, hypertension, and dyslipidemia, increasing the risk of cardiovascular disease [1]. The incidence of metabolic syndrome has risen in recent years, especially in developed countries, but there is currently no treatment [2].

Therapeutic strategies to prevent or reduce the consequences of these disorders are mainly based on diet and physical activity. In recent years, research has focused on exploring and using bioactive compounds with beneficial properties to handle chronic metabolic diseases [3]. Phenolic compounds and alkaloids are of particular interest among these bioactive compounds, which have been related to the prevention of diseases such as diabetes, obesity, or cancer, and exert neuroprotective, anti-inflammatory, and anti-hypertensive effects [3,4].

The cocoa shell is a by-product obtained from the cocoa industry during the processing of cocoa beans. The cocoa shell is removed from the outer portions of beans after the roasting process [5]. In cocoa production, the cocoa shell is considerable, representing 10–20% of the cocoa bean [6,7]. The cocoa shell is composed mainly of carbohydrates (7.8–70.2%), dietary fiber (39.2–66.3%), protein (10.3–27.4%), and fat (1.5–8.5%). The cocoa shell also contains other minority compounds like polyphenols (0.3 –9.5 %) and methylxanthines such as theobromine (0.4–1.8%) and caffeine (0.1–0.4%) [8]. Due to its high content of dietary fiber and valuable bioactive compounds (phenolic compounds and methylxanthines), the cocoa shell could be used as a new food ingredient with valuable potential in treating metabolic syndrome [9,10].

Network pharmacology is a new discipline that aims to understand the actions and interactions of drugs with multiple targets. This discipline is gaining popularity as a new tool in drug discovery for its cost-effectiveness as it uses *in silico* data and its good predictability. Network pharmacology combines multidisciplinary technologies, such as systems biology and computational biology, to build a complex network between phytochemicals and target disease biomarkers and elucidate the mechanism of active compounds in disease prevention [11,12]. Thus, this study aimed to investigate the cocoa shell's phytochemical composition and find the potential biological effects of its main components in preventing metabolic syndrome by using an integrated network pharmacology approach.

2. Materials and Methods

2.1. Phytochemicals Collection and Screening

The phytochemicals found in the cocoa shell were extracted from the literature through a wide-scale text-mining method.

2.2. ADME Screening

In silico methods for identifying the pharmacokinetics of the phytochemicals found in the cocoa shell were an effective approach for filtering among all active components. We employed two ADME parameters, including drug-likeness (DL) and oral bioavailability (OB), generated by three *in silico* ADME models. The compounds which successfully satisfy the criteria: DL ≥ 0.18 and OB ≥ 35% were treated as candidate compounds.

2.3. Target Fishing and Network Construction

2.3.1. Target Acquisition for Phytochemicals and Disease-Related Effects

Metabolic syndrome-related genes were searched and screened by the following electronic databases: GeneCard database (https://www.genecards.org/), OMIM database (https://www.omim.org/), PharmGkb database (https://www.pharmgkb.org/), Therapeutic Targets database (http://bidd.nus.edu.sg/BIDD-Databases/TTD/TTD.asp), and DrugBank database (https://www.drugbank.ca). The database search results were combined, and duplicate targets were deleted to obtain all the targets for preventing metabolic syndrome.

2.3.2. Construction of Active Phytochemicals and Disease Target Network

The screening results of the active phytochemicals of the cocoa shell in metabolic syndrome targets were imported into Cytoscape, and an interactive network was constructed.

2.3.3. Mechanism of Action Predicted for Major Phytochemicals in the Cocoa Shell

The mechanism of action of major compounds in the cocoa shell was predicted using Prediction of Activity Spectra for Substances (PASS). This tool provides quantitative structure-activity relationships containing >205,000 compounds based on the chemical structures in 2D and 3D descriptors and the generation of models obtained from bioactive ligands [13].

2.3.4. Protein-protein interaction network and enrichment analysis

Protein-protein interaction networks and enrichment analysis were carried out using Metascape. Each gene was studied for its pathway and process enrichment score for statistical significance of genes in each biological process. Genes were also clustered according to their pathways. The relationship between genes was also identified using the network map generated from the Metascape tool and visualized in the Cytoscape tool.

3. Results and Discussion

3.1. Methylxanthines and Phenolic Compounds are the Main Phytochemicals Found in the Cocoa Shell: The Biological Activity is Limited to their Different Bioavailability

The main phytochemicals found in cocoa shells (**Figure 1A**) were methylxanthines (theobromine (TH) and caffeine (CAF)) and phenolic compounds (protocatechuic acid (PCA), procyanidin B2 (PB2), (−)-epicatechin (EPI), and (+)-catechin (CAT)) [14]. According to the ADMET predictions, CAF was the compound showing the highest bioavailability (79.6%), followed by TH, CAT, EPI, PCA (65.7, 62.8, 54.2, 49.1%, respectively), although

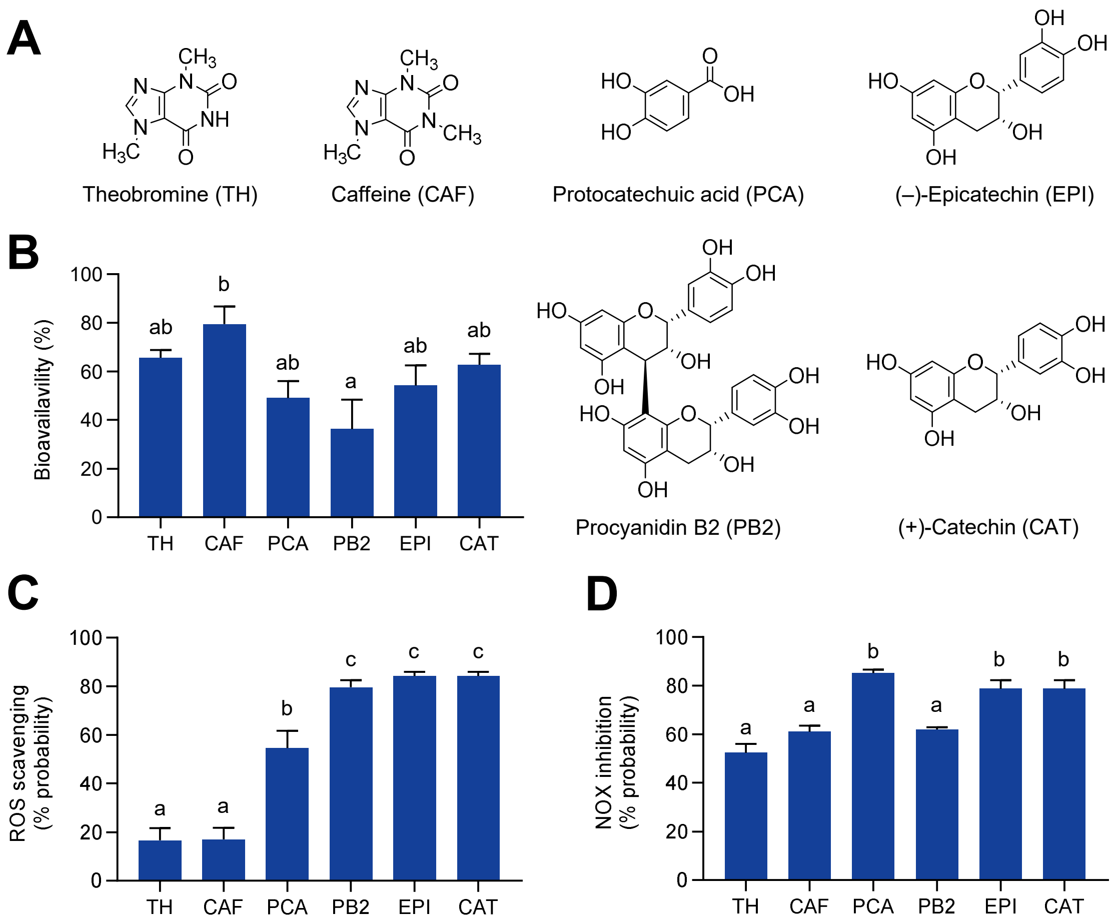

Figure 1. Chemical structure of the main phytochemicals found in the cocoa shell (**A**), their predicted bioavailability (**B**), and their potential to scavenge ROS (**C**) and inhibit NADPH oxidase (NOX) (**D**).

not showing significant ($p > 0.05$) differences among them. However, the compound with the lowest ($p < 0.05$) bioavailability was PB2 (36.2%). The bioavailability of phytochemicals is crucial to ensure these compounds' bioefficacy in modulating metabolic targets [15]; therefore, these six phytochemicals seem to be potential nutraceuticals candidates.

3.2. Phenolic Compounds from the Cocoa Shell exhibit Reactive Oxygen Species (ROS) Scavenging Properties and Regulates Oxidative Stress-Related Pathways

Phenolic compounds present in cocoa shells showed ROS scavenging properties (**Figure 1C**). PB2, EPI and CAT exhibited the highest ($p < 0.05$) ROS scavenging values (79.8–84.2%). PCA presented a significantly ($p < 0.05$) lower ROS scavenging (54.6%) compared to the other phenolic compounds. Phenolic compounds, because of their structure, have the ability to interact with ROS, preventing cell viability from being affected [16]. The enzyme NADPH oxidase (NOX) (**Figure 1D**), whose only function is to produce ROS can be inhibited according to PASS predictions by PCA, EPI, and CAT (61.9–85.2%). PB2, TH, and CAF (52.4–61.1%) were also able to inhibit NOX to a lesser ($p < 0.05$) degree. Phenolic compounds have an inhibitory effect on NOX enzyme activity, which may be a good strategy to combat oxidative stress [17]. Similarly, phytochemicals in the cocoa shell present the potential ability to stimulate antioxidant enzymes, such as SOD (10.2–43.6%) and CAT (13.1–52.3%). Recently, several studies have demonstrated the ability of cocoa shell extracts rich in phenolic compounds in the prevention of ROS production [9,10,18].

3.3. Inflammatory Signaling Cascades, TNF-α, NF-κB, and JNK are Regulated by the Phenolic Compounds Present in the Cocoa Shell

The probability of phytochemicals from the cocoa shell to modulate immunostimulation (**Figure 2A**) was significantly higher ($p < 0.05$) in methylxanthines than in the phenolic compounds. In contrast, **Figure 2B** shows that phenolic compounds (PCA, PB2, EPI, and CAT) exhibited higher ($p < 0.05$) TNF-α inhibitory potential (63.1%) than methylxanthines (TH, 24.1% and CAF, 30.9%). Regarding the inhibition of the NF-κB (**Figure 2C**),

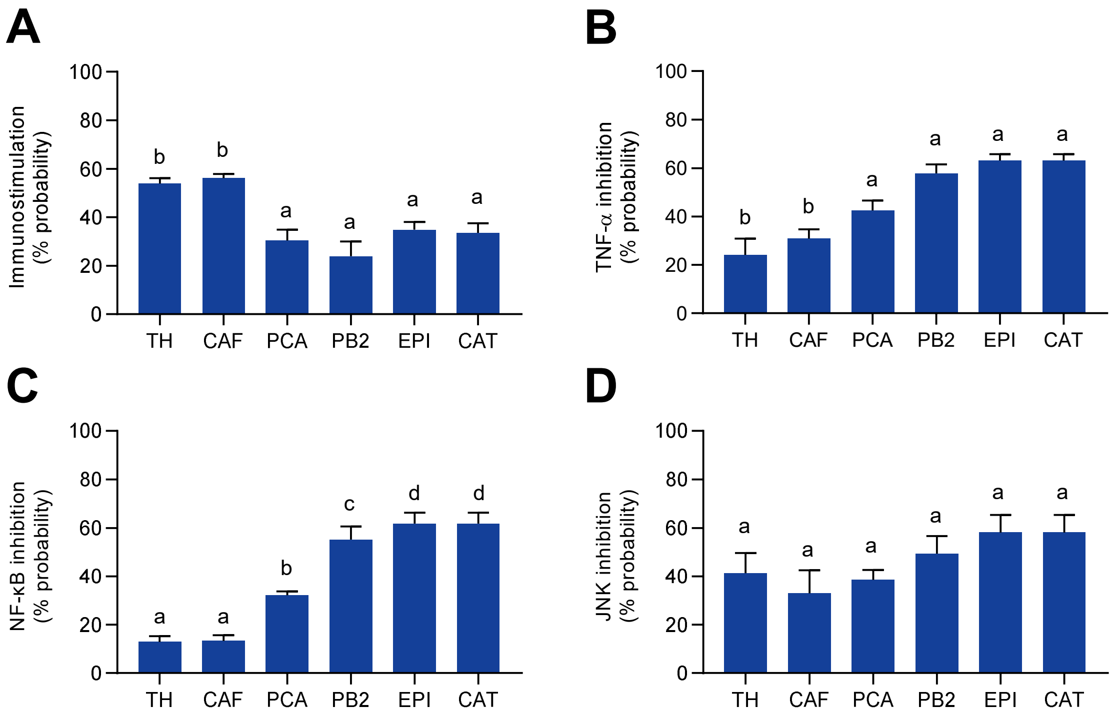

Figure 2. Potential effect of the phytochemicals from the cocoa shell on the modulation of immunostimulation (**A**), TNF-α production (**B**), and NF-κB (**C**) and JNK (**D**) signaling cascades activation.

EPI and CAT were the compounds that showed the highest inhibition capacity (61.8%), followed by PB2 (55.1%) and PCA (32.1%). All compounds were able to inhibit JNK (30.0–52.8%) (**Figure 2D**). TNF-α is one of the primary cytokines that stimulate inflammation. TNF-α can be inhibited by natural compounds, such as phenolic compounds, terpenes, and alkaloids, since these phytochemicals can interfere with pro-inflammatory mediators and upstream targets, such as NF-κB, JNK, and other signaling molecules, involved in TNF-α expression [19]. Moreover, since NF-κB and JNK are implicated in regulating the expression of many genes involved in obesity and diabetes-triggered inflammation, they represent a target in the search for new chemopreventive active compounds. In this regard, phenolic compounds may be promising due to their inhibitory effects on NF-κB and JNK activation.

3.4. Protocatechuic Acid is the Main Contributor to Glucose and Lipid Metabolism Regulation

PCA was significantly more likely and to inhibit the fatty acid synthase (FASN) (9.23% $p < 0.05$) and, in general, modulate lipid metabolism (71.0%, $p < 0.05$) than the other phytochemicals found in the cocoa shell (**Figures 3A-B**). Studies in animal models have shown an improvement in lipid profile after PCA consumption [20]. Methylxanthines, especially caffeine, showed a significant probability to regulate lipid metabolism (44.6–62.7% $p < 0.05$) but with a lower probability of inhibiting FASN (24.9–33.5% $p < 0.05$).

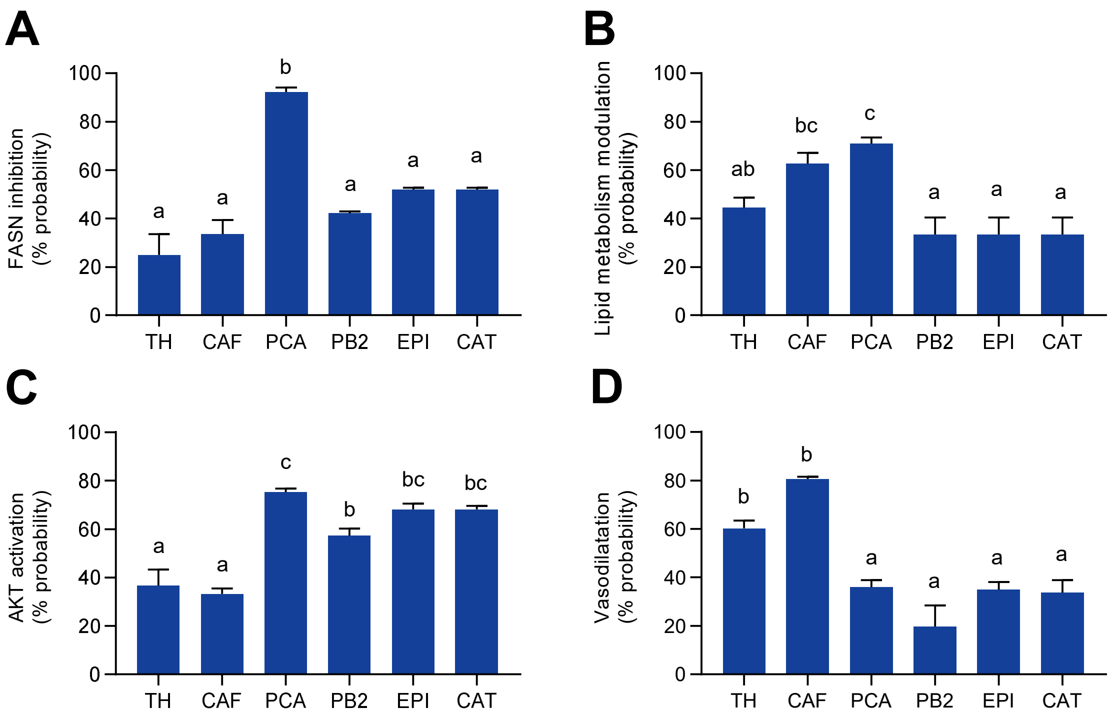

Figure 3. Regulatory properties of the phytochemicals from the cocoa shell on the inhibition of FASN (**A**), the modulation of lipid metabolism (**B**), the activation of protein kinase B (AKT) (**C**), and the stimulation of vasodilatation (**D**).

The main mechanism of action of methylxanthines in the regulation of lipid metabolism is through suppression of adipogenesis and stimulation of thermogenesis and lipolysis [21–23]. PI3K-AKT pathway has been reported as a target to reverse insulin resistance. The probability of activating AKT (**Figure 3C**) is higher ($p < 0.05$) in phenolic compounds (57.3–75.3%) than in methylxanthines (33.3–36.6%). PCA showed the greatest probability for activating AKT. Similarly, PCA exhibits insulin mimicking properties by activating INSR signaling, reversing INS-1 serine phosphorylation, and activating PI3K/AKT and AMPK signaling pathways, and subsequently increasing GLUT4 translocation and glucose uptake in adipocytes [24]. Besides, PCA has been shown to reduce glucose-6-phosphatase expression and activity and to promote glucose uptake in hepatocytes and myoblasts [25]. Hence, PCA seems to be a possible candidate in preventing metabolic disorders associated with lipid and glucose metabolism dysregulations and insulin resistance.

3.5. Methylxanthines, Theobromine and Caffeine, Present Vasoactive Properties

The methylxanthines found in the cocoa shell, especially caffeine (80.6%, $p < 0.05$), presented a high probability for vasodilatation (**Figure 3D**). Literature has evidenced that methylxanthines exhibit vasoactive properties. Caffeine can regulate vascular function through equilibrium between its endothelium-dependent vasodilator ability, mediated by the endogenous NO production and its vasoconstrictive effect as an adenosine receptor antagonist [26]. Notwithstanding the potential vasodilatation activity of theobromine (60.2%), it has only been reported to increase fasting and postprandial arterial diameter and decrease augmentation index in obese subjects, although not improving the endothelial function [27].

3.6. Cocoa Shell Phytochemicals Regulate Key Signaling Pathways Associated with the Prevention of Metabolic Syndrome

The phytochemicals present in the cocoa shell regulate key signal pathways associated with preventing metabolic syndrome (**Figure 4A, B**). Of particular interest is the prediction of these cocoa shell compounds and the possible regulation of pathways related to non-alcoholic fatty liver disease and insulin resistance and PI3K-Akt signaling pathways, as well as those related to lipid metabolism, adaptive thermogenesis, adipocytokine signaling, and cAMP signaling pathway, being key markers in the prevention of metabolic syndrome. These compounds also showed a significant relation with longevity regulating pathways, maybe due to the possible regulation observed in response to oxidative stress and mTOR pathways, closely related to cellular aging. In general, it is observed that the phytochemicals from the cocoa shell can modulate the main genes associated with metabolic syndrome and its complications. Results demonstrate that both methylxanthines and phenolic compounds exhibit biological effects following different molecular mechanisms.

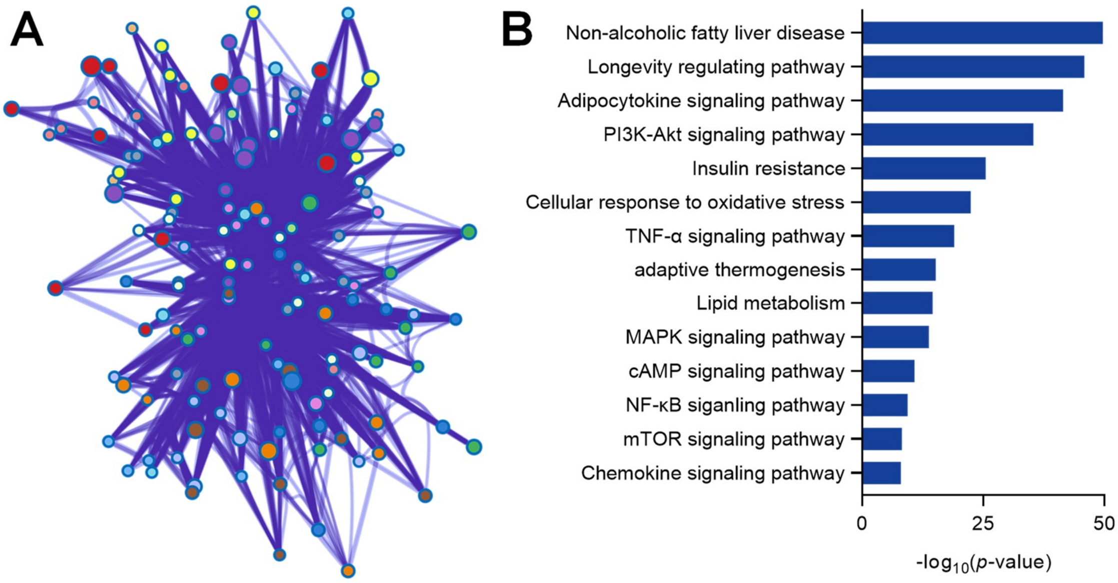

Figure 4. Protein-protein interaction networks built with Metascape from the most significant target genes associated with metabolic syndrome potentially modulated by the phytochemicals found in the cocoa shell (**A**) and the KEGG pathways associated with those proteins obtained by the enrichment analysis (**B**).

Therefore, using the cocoa shell to produce active ingredients focused on preventing chronic metabolic diseases will serve as a multimechanistic chemopreventive treatment. Our integrated network pharmacology analysis presents new insights into the biological activity and molecular mechanisms of the cocoa shell's main bioactive components, thereby establishing this by-product as a prospective agent for more *in vitro* and *in vivo* investigation, especially focused on the evaluation of its use for treating disorders involving oxidative stress, inflammation, glucose and lipid metabolism, and insulin resistance.

4. Conclusions

Results derived from this first integrated network pharmacology study demonstrated that cocoa shell phytochemicals could serve as modulators of key biological processes and signaling cascades associated with the prevention of metabolic syndrome. The revalorization of the cocoa shell as a nutraceutical could result in a product used for the dietetic prevention of chronic metabolic disorders. Upcoming *in vivo* and clinical investigations are required to validate this potential nutraceutical and healthy ingredient's effects and mechanisms.

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preparation, M.R.H., S.C., and C.B.; writing-review and editing, M.R.H., S.C., C.B., S.M.A., and M.A.M.C.; visualization, M.R.H.; supervision, M.R.H. and M.A.M.C.; funding acquisition, S.M.A. and M.A.M.C. All authors have read and agreed to the published version of the manuscript.

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