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REVIEW ARTICLE

A Therapeutic Connection between Dietary Phytochemicals and ATP Synthase

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Abstract: For centuries, phytochemicals have been used to prevent and cure multiple health ailments. Phytochemicals have been reported to have antioxidant, antidiabetic, antitussive, antiparasitic, anticancer, and antimicrobial properties. Generally, the therapeutic use of phytochemicals is based on tradition or word of mouth with few evidence-based studies. Moreover, molecular level interactions or molecular targets for the majority of phytochemicals are unknown. In recent years, antibiotic resistance by microbes has become a major healthcare concern. As such, the use of phytochemicals with antimicrobial properties has become pertinent. Natural compounds from plants, vegetables, herbs, and spices with strong antimicrobial properties present an excellent opportunity for preventing and combating antibiotic resistant microbial infections. ATP synthase is the fundamental means of cellular energy. Inhibition of ATP synthase may deprive cells of required energy leading to cell death, and a variety of dietary phytochemicals are known to inhibit ATP synthase. Structural modifications of phytochemicals have been shown to increase the inhibitory potency and extent of inhibition. Site-directed mutagenic analysis has elucidated the binding site(s) for some phytochemicals on ATP synthase. Amino acid variations in and around the phytochemical binding sites can result in selective binding and inhibition of microbial ATP synthase. In this review, the therapeutic connection between dietary phytochemicals and ATP synthase is summarized based on the inhibition of ATP synthase by dietary phytochemicals. Research suggests selective targeting of ATP synthase is a valuable alternative molecular level approach to combat antibiotic resistant microbial infections.

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1. INTRODUCTION

Antimicrobial resistance is becoming an existential threat to mankind. According to *The Review on Antimicrobial Resistance* [1] antibiotic resistance will result in about ten million additional deaths per year worldwide by 2050. Currently, more than 700,000 people die from microbial infections every year. Thus, antibiotic-resistant microbes are expected to become the top global killers, surpassing cancer. The impact of this public health crisis on the global economy is projected to be about $100 trillion [2]. Furthermore, bacteria keep evolving so that they can resist the new drugs that are used to combat them. This fast-encroaching antibiotic resistance has become particularly problematic in recent years because the discovery of new antibiotics has not kept pace. Finally, this problem is not limited to bacteria because all microbes that have the potential to mutate can make widely used drugs ineffective [1].

Antibiotic resistance threatens the prevention and treatment of infections caused by bacteria, parasites, viruses, and fungi. *Klebsiella pneumonia*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Neisseria gonorrhoeae*, in general, and *Escherichia coli*, in particular, are the main reasons for this alarming situation [3-5]. *E. coli*, a naturally resistant, gram-negative bac-

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terium with a complex cell wall barrier against several drugs, is at the forefront of drug resistance [6]. Finding new, alternative ways to kill microbes is of paramount importance. In this review, we reiterate the therapeutic link between antimicrobial properties of dietary phytochemicals and energy-generating ATP synthase as a potent molecular drug target.

2. ATP SYNTHASE

The enzyme, ATP synthase (EC 3.6.3.14), is important for the normal physiological function of cells because it is the principal energy-generating nanomotor of cells. Structurally, ATP synthase is very similar in almost all organisms from bacteria to man. In its simplest form, bacterial ATP synthase has two sectors, a water-soluble F_1 sector and a membrane-embedded F_o sector. The F_1 sector is composed of five subunits $\alpha_3\beta_3\gamma\delta\varepsilon$, where the catalytic activity occurs; and the F_o sector has three subunits from which protons are pumped (Fig. **1**). In the catalytic subunits, the cyclical process known as the binding change mechanism generates adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and inorganic phosphate (Pi) [7].

Mammalian mitochondrial F_1F_o ATP synthase is slightly more complex than the bacterial ATP synthase, with additional subunits and different names. For example, δ subunit of mammalian ATP synthase is homologous to the ε subunit of *E. coli.* A subunit called oligomycin sensitivity conferral protein of mammalian ATP synthase is homologous to the δ subunit of *E. coli* ATP synthase. Mammalian ATP synthase also has two additional subunits d and F_6 [8, 9]. Additionally, there are multiple variations in amino acid types and positions in all subunits. These variations may play a critical role in selective binding of inhibitors [10-13].

The cytoplasmic concentrations of ATP and Pi in the active cells are in the range of 2-5 mM, whereas that of ADP is at least 10-50-fold lower. Equilibrium binding assays have established that both ADP and ATP bind to catalytic sites of F_1F_o ATP synthase with relatively similar binding affinities [14-18]. With such unfavorable low physiological concentration of ADP in the cells, the designated catalytic site amino acids in the α/β interface of ATP synthase bind to Pi and support the ADP and Pi interaction to form ATP [19]. ATP thus formed is the fundamental means of cellular energy. Inhibition of ATP synthase will cease the ATP formation depriving cells of vital energy. Dietary phytochemicals and other inhibitors cause variable degree of ATP synthase inhibition. The extent of inhibition is

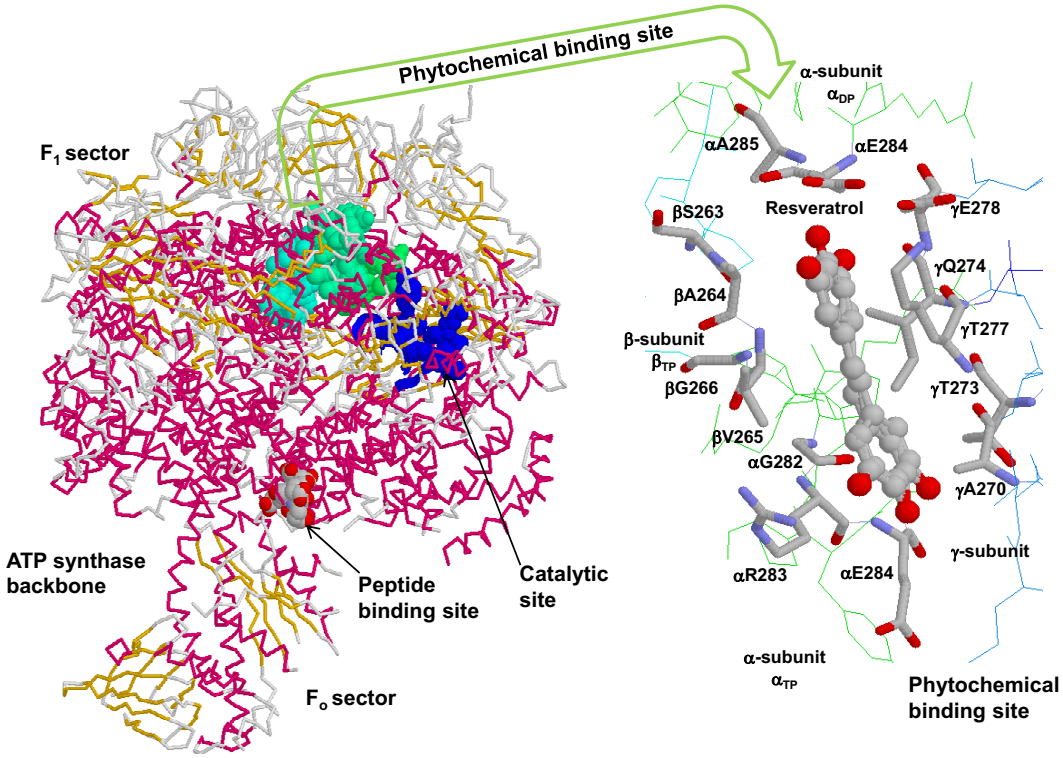

Fig. (1). *Backbone form of F_1F_o ATP synthase with phytochemical binding site.* The F_1 sector of the enzyme shows the catalytic Pi (phosphate) binding subdomain, peptide, and phytochemical binding sites in space fill form. The resveratrol bound phytochemical binding site contributed by α-, β-, and γ-subunit residues is zoomed in wireframe form. Figure was taken from references [11, 28] and was generated by PDB files 1H8E [146] and 2JIZ [10] using Rasmol [147].

directly proportional to the molecular level interactions which depend on the interaction between functional groups of inhibitors and binding site amino acids of ATP synthase. The molecular-level interactions between inhibitors and binding-site residues have been resolved for some and are under investigation for many [10-12, 20-25].

3. ATP SYNTHASE UNDER DISEASE CONDITIONS

ATP synthase is vital to human health. Malfunction of this energy-generating enzyme complex has been associated with a variety of pathological conditions [20, 26], such as hypertension, alcoholism, cardiovascular diseases, cancer, tuberculosis, neuropathy, Alzheimer's disease, Parkinson's disease, Down's syndrome, neuronal diseases, aging, immune responses, uteroplacental insufficiency, albinism and mitochondrial myopathies [20, 26-28]. Multiple studies have linked the alteration of F_1F_o ATP synthase to these disease conditions [26]. For instance, alcohol is known to severely damage the mitochondria. With chronic alcoholism, production of ATP by F_1F_o ATP synthase declines in the liver and brain [29]. In one study, the cysteine and tyrosine residues of α- and β-subunits of F_1F_o ATP synthase were oxidized in rats subjected to prolonged ethanol exposure, resulting in decreased mitochondrial ATP production [30]. In diabetes, F_1F_o ATP synthase also plays a significant role. Compromised F_1F_o ATP synthase has been shown to result in diminished ATP production, which affects insulin secretion and glucose uptake [31]. Further, the phosphorylation of βTyr-361 and βThr-213 has been shown to be responsible for the down regulation of β-subunit F_1F_o ATP synthase, and the ATP synthase β-subunit amino acids from the skeletal muscles of type 2 diabetic and obese patients have shown pronounced phosphorylation at multiple sites [32].

ATP synthase has been linked to high blood pressure. High levels of circulating subunit F6 have been observed with hypertension, suggesting an association between ATP synthase and high blood pressure [33, 34]. The F_1F_o enzyme has also been implicated in autoimmune disease conditions, such as systemic lupus erythematosus (SLE). For instance, the ATP depletion in T-cells and defective DNA repair mechanisms may contribute to the autoimmune pathology of SLE. A comparative DNA micro array of healthy and SLE human patients showed down regulation of A6L and *a*-subunit of ATP synthase [35]. In maternally inherited Leigh syndrome, a neurodegenerative disease and neuropathy, ataxia, the T8993G mutation in the *a*-subunit causes a severe impairment and dysfunction of ATP synthase [36-39]. Human neuropathological conditions have been documented with mutations in the mitochondrial genome with T8993G or T8993C causing energy depletion or mitochondrial reactive oxygen species production, respectively. These mutations were found to disrupt the ATP synthase coding for *a*Leu-156 [37]. Abnormal lysosomal ATP synthase *c*-subunit accumulation has been observed in Batten disease, a neurodegenerative fatal disease of humans and other animals [40].

Aging also affects the activity levels of F_1F_o ATP synthase subunits. With age, an increase in the expression of α-, β-, and *d*-subunits was observed in rat skeletal muscle [41] and an increase in the expression of F-subunit was observed in mouse brain [42]. Age-induced decreases in α - and β-subunits have been documented in mouse heart [43] along with decreased ATPase activity in aging rat kidneys [44]. One of the age-related mitochondrial defects is a 4977 base pair deletion. This deletion negatively affects the biological oxidation of electron transport chain complexes nicotinamide adenine dinucleotide dehydrogenase, cytochrome oxidase, and ATP synthase [45]. In another study, age-related ATP synthase changes in skeletal muscle have been observed, resulting in decreased ATP production per mole of O_2 consumed [46]. In Alzheimer's disease or presenile dementia, a deficiency of mitochondrial ATP synthase was reported [47]. A low expression of β-subunit and buildup of the α-subunit have been noted in Alzheimer's disease, and ATP synthase α-subunit buildup in the intraneuronal cytosole is associated with the neurodegenerative process [48-50]. Moreover, ectopic ATP synthase on the cell surface of endothelial cells is implicated in the angiogenesis process that is essential for tumor growth [51-54].

4. ATP SYNTHASE AS A MOLECULAR DRUG TARGET

ATP synthase is also being used and recommended as an effective molecular drug target for multiple disease conditions and for the regulation of energy metabolism ([20, 26, 55, 56] and references therein). Selective and specific inhibition makes ATP synthase an excellent molecular target for the development of new antimicrobial agents. For example, the antituberculosis drug Bedaquiline, approved by the US Food and Drug Administration in 2012, is highly selective in its inhibition of the F_o sector of ATP synthase in mycobacterial species [2, 57-61]. Bz-423 a drug for the autoimmune

disorder systemic lupus erythematosus selectively kills pathogenic lymphocytes by inducing apoptosis in lymphoid cells [62]. Bz-423 inhibits the mitochondrial ATP synthase by binding the subunit OSCP the oligomycin sensitivity-conferring protein [63, 64].

Apoptolidin, a selective cytotoxic agent, is an inhibitor of F_1F_o ATP synthase. Apoptolidin is a macrolide originally isolated from the *Nocardiopsis* species and selectively kills E1A and E1A/E1B19K transformed rat glial cells (IC_{50} = 11 ng/ml) while not killing untransformed glial cells [65]. Apoptolidin is among the most selective cytotoxic agents tested by the National Cancer Institute in human cancer cell lines. The apoptotic mechanism of action of apoptolidin is through selective inhibition of F_1F_o ATP synthase [66, 67]. Selective ATP synthase inhibition has been attributed to the type and position of amino acids in and around the inhibitor binding sites [22, 25]. Slight variation in amino acids was shown to cause selective and specific inhibition of ATP synthase from a variety of food sources. For example, tentoxin strongly inhibits F_1-ATPase in spinach, potato, and lettuce but causes no inhibition of the same enzyme from species, such as corn and radish, even though they exhibit high sequence and structural similarity [68-70].

5. ECTOPIC ATP SYNTHASE

The inner membrane of the mitochondria was considered the exclusive location of F_1F_o-ATP synthase [19]. However, multiple studies have documented the occurrence of ectopic F_1F_o-ATP synthase on cell membranes. The actual protein transport mechanism of the ectopic ATP synthase is not clear. The green fluorescent protein-ATP5B fusion introduced into HepG2 cells to study the localization of the ATP synthase suggested that the ectopic expression of ATP synthase occurs from the translocation of mitochondrial ATP synthase [71].

Targeting of ectopic ATP synthase inhibits cytosolic lipid droplet buildup, making ATP synthase a potential molecular target for antiobesity drugs [72]. Ectopic ATP synthase serves as a ligand receptor and participates in numerous cellular processes, such as angiogenesis, lipid metabolism, and the cytolytic pathway of tumor cells. Inhibition of ATP synthase blocks tumor angiogenesis, making it a suitable antiangiogenic therapeutic target [51, 54, 73-77]. Inhibition of ectopic ATP synthase prominently obstructs the migration and proliferation of endothelial cells with little effect on intracellular ATP [75]. On the surface of endothelial cells ectopic ATP synthase β-subunit activates cytotoxic activity by attracting inflammatory cells [78]. Additionally, ectopic ATP synthase was also identified to mediate HIV-1 transfer between antigen-presenting cells and $CD4^+$ target cells [79].

6. ATP SYNTHASE INHIBITORS

More than 300 natural and synthetic molecules are known to bind and inhibit ATP synthase. The interaction between the majority of these inhibitors and ATP synthase residues and the specific sites remains unknown [10, 20, 80]. The two therapeutically important antimicrobial ATP synthase inhibitors are antimicrobial peptides, which mainly bind at the peptide-binding pocket formed by the βDELSEED-motif [13, 81-88], and antimicrobial phytochemicals, which mainly bind at the phytochemical or polyphenol binding pocket contributed by α -, β-, and, γ -subunit residues [10-12, 21-24, 80, 88-103]. Selective inhibition of F_1F_o ATP synthase is a promising way to deal with multiple disease conditions, including antibiotic-resistant microbial infections.

7. PHYTOCHEMICALS AS INHIBITORS OF ATP SYNTHASE

A wide range of phytochemicals are known to have antimicrobial properties [4, 5]. Many of them have been shown to inhibit bacterial ATP synthase (Figs. **2** and **3**). Thymoquinone [21], safranal [22], piceatannol [11], and baicalein [12] induced complete inhibition of *E. coli* wild-type F_1F_o ATP synthase as shown in Fig. (**2**). The Table **1** lists some of the antimicrobial phytochemicals that inhibit bacterial (*E. coli*) ATP synthase to variable degrees. Potency and the extent of inhibition on a molar scale differ among the various inhibitors. Further, the extent of ATP synthase inhibition depends on the type and positioning of the functional groups of phytochemicals [89]. Thus, addition, deletion, and rearrangement of functional groups can enhance the degree of inhibition. For example, as shown in (Fig. **3**), natural resveratrol causes about 40% inhibition of ATP synthase with IC_{50} at about 94 μM. Structural modulation of resveratrol by removal, addition, or repositioning of its functional groups resulted in 100% inhibition with IC_{50} values from about 94 μM to about 2.50 μM [11, 89]. Another phytochemical, hydroxytyrosol from olives, caused about 60% inhibition of *E. coli* membrane-bound F_1F_o ATP synthase. Structural modifications with the repositioning of its -OH groups resulted in almost 100% inhibition (Z. Ahmad unpublished data).

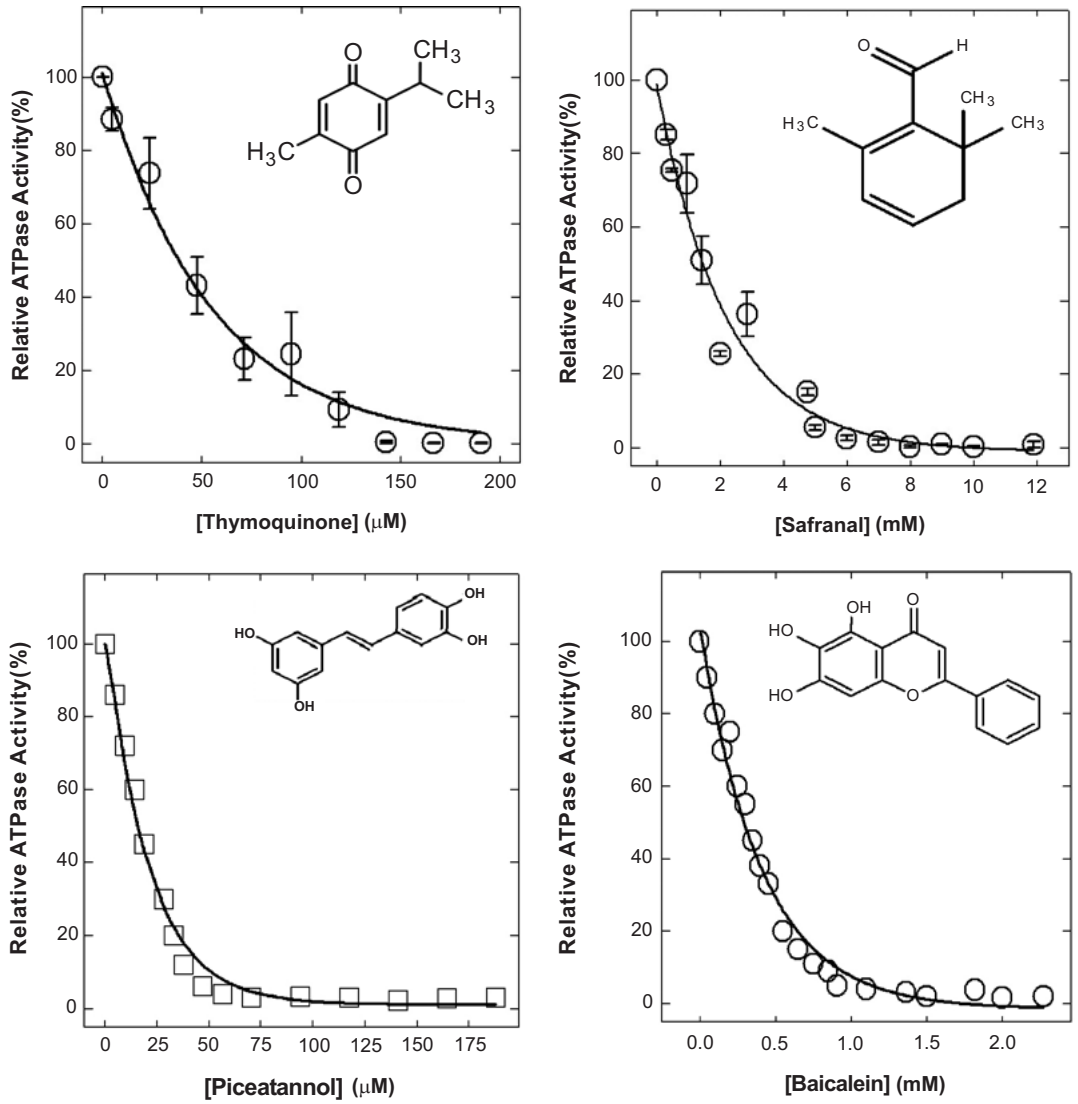

Fig. (2). *Phytochemical induced inhibition of F_1F_o ATP synthase.* Thymoquinone, safranal, piceatannol, and baicalein induced inhibition of *E. coli* wild-type F_1F_o ATP synthase. Figure compiled from references [11, 12, 21, 22].

Table 1. Phytochemical induced inhibition of bacterial wild-type F_1F_o ATP synthase.

Phytochemicals	Maximal inhibition (~ %)	Estimated IC_{50} values (μM)	Reference source (references number)
Resveratrol	40	N/A	[11]
Piceatannol	100	16	[11]
Quercetin	80	N/A	[11]
Quercitrin	40	N/A	[11]
Quercetin-3-β-D glucoside	50	N/A	[11]
Morin	100	70	[12]
Silymarin	100	110	[12]
Baicalein	100	290	[12]
Amantadine	100	2500	[12]
Rimantadine	100	2000	[12]

(Table 1) contd….

Phytochemicals	Maximal inhibition (~ %)	Estimated IC_{50} values (μM)	Reference source (references number)
Epicatechin	100	4000	[12]
Silibinin	100	340	[12]
Hesperdin	60	N/A	[12]
Apigenin	40	N/A	[12]
Diosmin	50	N/A	[12]
Chrysin	60	N/A	[12]
Rutin	40	N/A	[12]
Kaempferol	58	N/A	[12]
Genistein	40	N/A	[12]
Galangin	0	N/A	[12]
Luteolin	20	N/A	[12]
Daidzein	10	N/A	[12]
Hydroquinone	80	N/A	[89]
Dihydrothymoquinone	40	N/A	[89]
Resorcinol	20	N/A	[89]
Catechol	40	N/A	[89]
Modified resveratrol 1 (MR1)	100	2.5	[89]
Modified resveratrol 2 (MR2)	100	7	[89]
Thymoquinone	100	38	[21]
Safranal	100	1600	[22]
Thymol	87	N/A	[22]
Carvacrol	100	2800	[22]
Damascenone	85	N/A	[22]
Cuminol	93	N/A	[22]
4-Kettoisophorone	90	N/A	[22]
Curcumin	60	N/A	[24]
Tyrosol	100	9500	[25]
Hydroxytyrosol	62	N/A	[25]
Dihydroxyphenylglycol	35	N/A	[25]
Oleuropein	40	N/A	[25]
Theaflavin (TF1)	85	N/A	[23]
Theaflavin-3-gallate (TF2A)	95	N/A	[23]
Theaflavin-3′-gallate (TF2B)	95	N/A	[23]
Theaflavin-3,3′-digallate (TF3)	90	N/A	[23]
Epigallocatechin gallate (EGCG)	95	N/A	[23]

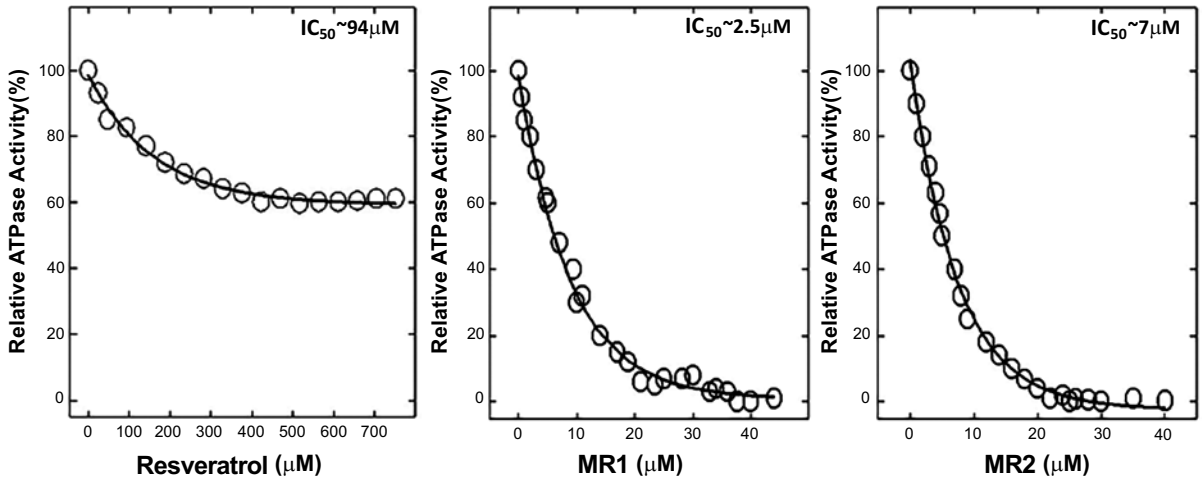

Fig. (3). Inhibitory effect of resveratrol and structurally modified resveratrol containing nitro groups hydroxyl-nitrophenyl-imino methylphenol (MR1 & MR2). Structural modification caused 100% inhibition and reduced IC_{50} from 94 μM to 2.5 μM and 7 μM. Figure taken from reference [89].

8. SIGNIFICANCE AND RATIONALE OF DIETARY PHYTOCHEMICALS AS INHIBITORS

Phytochemicals are naturally occurring plant-based compounds that possess antimicrobial, chemopreventive, and antioxidant properties [10, 23, 104-106]. Foods such as apples, berries, cherries, grapes, pears, plums, dates, cantaloupe, ginger, turmeric, garlic, onions, broccoli, olives, and saffron are rich in phytochemicals with antimicrobial properties [107-119]. Phenolic compounds are one of the important classes of phytochemicals shown to block the action of enzymes and other substances that promote the growth of cancer [120-123] and microbial cells [124-128].

Physiological relevance of dietary phytochemicals can be ascribed to their interaction with mitochondrion in eukaryotic cells. Throughout the aging process, many degenerating diseases and neurological disorders are attributed to mitochondrial dysfunction [38]. Thus, the selective inhibition of ATP synthase by natural or structurally modified phytochemicals might play a significant role in the physiology of such conditions [10, 12, 23, 89, 129, 130]. Grape constituent resveratrol, a dietary phenolic phytochemical, is widely known for its chemopreventive actions [121, 122, 131-135] and, in some cases, it has induced apoptosis *via* mitochondrial pathways [27, 132]. Earlier [136], oligomycin, a highly specific ATP synthase inhibitor, was shown to induce an apoptotic suicide response in cultured human lymphoblastoid and other mammalian cells within 12-18 hours but not in ρ^o cells that are depleted of a functional mitochondrial respiratory chain. A similar study [137] suggested that inhibition of the components of mitochondrial pathways may lead to the marking of some cells, *via* CD14, for cell death, whilst allowing differentiation to occur in the surviving population. Thus, there is possibility of targeting tumor cells without affecting the normal cells through selective inhibition of ATP synthase by phenolic phytochemicals [10-12, 23].

For centuries, dietary phytochemicals have been used worldwide as household remedies for multiple ailments, including antimicrobial agents [116, 138-141]. Multiple studies have linked the antimicrobial actions of dietary phytochemicals to the inhibition of bacterial F_1F_o ATP synthase [11, 12, 23, 27]. Biological activity against *Streptococcus mutans* is an excellent example. *S. mutans* is a primary microbial agent in the pathogenesis of dental caries, and dietary phenolic phytochemicals have been shown to inhibit biofilm formation and acid production by *S. mutans*. One of the pathways through which phytochemicals are active against traits of *S. mutans* is the selective inhibition of its proton-translocating ATP synthase activity [130]. Thus, further understanding of the mechanism of inhibition of ATP synthase by natural, synthetic, and structurally modified dietary phytochemicals has a potential to develop better strategies for combating drug-resistant bacteria.

Research suggests that the beneficial effects of dietary phytochemicals are linked to the blocking of ATP synthesis in tumor and bacterial cells [10, 12, 22]. Many dietary phytochemicals, such as safranal, resveratrol, piceatannol, morin, silymarin, baicalein, silibinin, rimantadin, amantidin, epicatechin, and

theaflavin, bind and inhibit *E. coli* ATP synthase [11, 12, 22, 23]. Further, the wild-type (pBWU13.4/DK8) *E. coli* growth was fully abrogated, and little or no growth inhibition occurred in the null (pUC118/DK8) *E. coli* strain with the deleted ATPase gene in the presence of dietary phenolic compounds [11, 12, 21, 22, 24]. In the absence of ATP synthase, the little loss of growth among null cells could be the result of action on other possible targets. The damage to the cell membrane by destabilization or permeabilization, inhibition of other microbial enzymes, or blockage of essential substrates, such as iron or zinc, required for microbial growth are possible [26, 96, 142-145]. Likewise, the total loss of growth in wild-type *E. coli* can be attributed to the inhibition of ATP synthase along with other targets [13].

9. SIGNIFICANCE OF MUTAGENIC ANALYSIS OF INHIBITOR-BINDING SITES AND SURROUNDING RESIDUES OF ATP SYNTHASE

To generate structurally and functionally potent antibacterial phytochemicals, it is vital to understand their interaction with binding sites and the surrounding residues. Resveratrol-, piceatannol-, and quercetin-bound x-ray structures suggest that α-, β-, and γ-subunit residues αG282, αR283, αE284, αA285, βS263, βA264, βV265, γA270, γT273, γQ274, γT277, and γE278 may be directly involved in polyphenol binding [10]. Moreover, about 30 plus amino acids closely flank the phytochemical binding pocket, and so far their role has not been deciphered. The side chains of these residues protrude directly into the phytochemical binding pocket. Therefore, the flanking residues may have some role in the binding and orientation of phytochemicals.

A majority of the phytochemical binding and surrounding residues are highly conserved throughout the evolution but do have some species or organismal level variations. There are more than 50 variations in and around the phytochemical binding pocket of *E. coli* and *Homo sapiens* (human*)* ATP synthase [10-12]. Moreover, there is a stretch of 11 amino acids between α311 and α321 omitted in human ATP synthase that encloses the polyphenol binding site of *E. coli*. These variations may provide the basis for selective inhibition of bacterial ATP synthase. These differences may also help in modifying the functional groups of inhibitors to make them more selective and potent. Charge and mass of the inhibitor binding site and its surrounding residues play a critical role in the binding and interaction with phytochemical functional groups. Therefore, mutagenic analysis of the inhibitor binding site and surrounding residues is pivotal for elucidating the action and selective interaction of phytochemicals with the inhibitor binding site.

Recently mutagenic analysis of phytochemical binding site residues has allowed us to specify the residues required for the binding and inhibition of safranal and tyrosol [22]. For example, αArg-283 plays critical role and is required for the binding of safranal and tyrosol, while residues αGlu-284, βVal-265, and γThr-273A play a less important role in binding and inhibition by safranal and tyrosol [22]. Moreover, both F_1 and F_o sector subunit residues have been identified and documented as contributing to binding sites for phytochemicals [10, 20, 26, 28, 55].

CONCLUSION

Phytochemical induced inhibitory studies of wild-type, mutant, and null *E. coli* ATP synthase suggest that the antimicrobial properties of dietary phytochemicals can be linked to the inhibition of bacterial ATP synthase. Selective inhibition of ATP synthase provides an alternative way to combat antibiotic-resistant microbial infections. Mutagenic analysis of the phytochemical binding site(s) can help identify selective and potent inhibitors. Functional groups on the dietary phytochemicals are critical for apt and effective inhibition. The potency and degree of inhibition of ATP synthase can be amplified by structural modulations, additions, and subtractions of functional groups on phytochemicals. Moreover, organismal and species level variations in the residues flanking the conserved phytochemical binding site can be utilized to identify the selective and potent microbial inhibitors.

LIST OF ABBREVIATIONS

ADP	=	Adenosine diphosphate
ATP	=	Adenosine triphosphate
DNA	=	Deoxyribonucleic acid
Pi	=	Inorganic phosphate
ROS	=	Reactive oxygen species
SLE	=	Systemic lupus erythematosus

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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