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Original Article

In vitro Screening for Antioxidant and Antimicrobial Properties of 3,5-Bis(E-thienylmethylene) piperidin-4-one, a Curcumin Analogue

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ABSTRACT

Background: Curcumin is a naturally occurring bis-chalcone derivative in the commonly used Indian spice turmeric and is well-known for its potent chemopreventive, anti-angiogenic, anti-cancer properties. 3,5-bis[(E)-thienylmethylene] piperidin-4-one (BTMP) is the newly synthesised synthetic analogue of curcumin that is tested for its antioxidant and antimicrobial properties *in vitro*. **Objectives:** This study was to determine the antioxidant activity and antibacterial potentials of newly synthesised analogues of curcumin *viz.*, 3,5-bis[(E)-thienylmethylene] piperidin-4-one (BTMP), was examined *in vitro*. **Materials and Methods:** BTMP was evaluated for its antioxidant and antimicrobial using 1,1-diphenyl-2-picryl hydroxyl (DPPH$^{\bullet}$) radical scavenging assay, 2,2′-azinobis3-ethyl benzothiazoline-6-sulfonic acid (ABTS$^{\bullet+}$), ferric reducing antioxidant power (FRAP), nitric oxide scavenging assay, superoxide anion radical scavenging assay ($O2^{\bullet-}$), hydrogen peroxide scavenging, reducing ability assay, metal ion chelating and phosphomolybdenum assays. **Results:** 40µg/ml of BTMP showed higher antioxidant activity than the other three doses due to its highest free radical scavenging ability at this particular dose. All doses of BTMP showed antimicrobial activity against *E. coli* and *S. aureus*. 40µg/ml of BTMP showed the highest zone of inhibition against both tested pathogens. **Conclusion:** The introduction of basic functionality and the presence of exocyclic double bonds of BTMP has significantly contributed to the antioxidant activity and antimicrobial activity against both gram-positive and gram-negative bacteria.
Keywords: Antioxidant, Antibacterial, Curcumin, DPPH, Reducing ability.

INTRODUCTION

Chalcones, or 1,3-diaryl-2-propen-1-ones, are one of the major classes of natural products with widespread distribution in fruits, vegetables, and spices.[1] They are precursors of flavonoids and isoflavonoids and have been found to possess interesting pharmacological properties.[2-3] These α, β-Unsaturated ketones being unique templates, besides being synthetically more important, are also associated with several biological activities.[4] It has been extensively reported that a vast number of naturally occurring chalcones and bis-chalcone derivatives, possessing the cal quenching properties, have raised interest in using these compounds like drugs and food preservatives.[5] The cytotoxic effects of 2,6-bis(arylidene) cyclohexanones revealed that these are more potent than clinically useful drugs. Also, they are versatile and convenient intermediates for the synthesis of a variety of heterocyclic compounds.[6] Hence, chalcones and their analogues are of interest from both biological and chemical points of view.

Curcumin is a naturally occurring bis-chalcone derivative present in the commonly used Indian spice turmeric and well-known for its potent chemo-preventive, anti-angiogenic, anti-cancer properties.[7-9] BTMP is the synthetic analogue of curcumin and structurally the combination of piperidin-4-ones with arylidene moieties. Piperidin-4-ones have been shown to possess antiviral, antitumor,[10] analgesic,[11] local anesthetic,[12] herbicidal,[13] antimicrobial,[14] fungicidal,[15] insecticidal, antihistaminic,[16] anti-inflammatory,[17] CNS stimulant,[18] anti-cancer,[16] and antituberculosis activities.[19] Interestingly, it has also been reported that the torsion angles of the arylidene aryl rings and topography of the substituent on N-atom of piperidin-4-one have contributed to the pharmacological properties.[20]

Furthermore, increased amounts of free radicals, are exceedingly hazardous to the organisms. When electron movement and energy production become uncoupled, increases the formation of oxygen free

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radicals, which, can cause diseases.[21] Due to weaker cellular antioxidant defense systems, and increased free radical formation caused cell damage may be responsible for many diseases and disorders including cardiovascular diseases, diabetes, inflammatory disorders, viral infections, and neurological ailments.[22] The medical goal of managing these illnesses without adverse effects remains a problem. As a result, there is a growing interest in using phytochemicals to improve medications.[23] Various laboratories throughout the world are working to develop drugs with low-cost, high-potential, and free of adverse effects.[24] Butylated hydroxytoluene (BHT), butylated hydroxy anisole (BHA), tertiary butylated hydroquinone, and gallic acid esters are synthetic antioxidants that have been linked to liver damage and cancer.[25] As a result, the importance of locating and utilising natural antioxidants, particularly those derived from plants, has risen dramatically in recent years.[26] Natural compounds are gaining popularity as potential antioxidants. Natural products have long been utilised in traditional medicine and have shown promise as a source of components for the development of novel pharmaceuticals.[27]

Hence, the efforts have been focused on one such compound BTMP, and *in vitro* studies on antioxidant and antimicrobial activity have been done in the present study.

MATERIALS AND METHODS

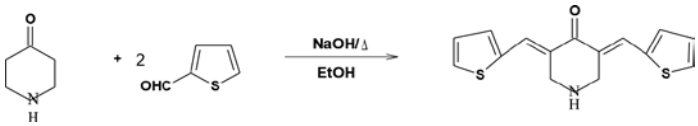
Reagents

Piperiridin-4-one hydrochloride, thiophene-2-carboxaldehyde, were purchased from Sigma-Aldrich. The aldehydes were used after distillation.

DPPH and butylated hydroxytoluene (BHT) (St Louis, MO, USA) are purchased from Sigma Aldrich. Sodium carbonate, sodium phosphate, potassium acetate, ethylene diamine tetra acetic acid, methanol, ethyl acetate, chloroform, sulphuric acid, trichloroacetic acid (TCA), and hydrogen peroxide reagents (Mumbai) were purchased from Qualigens. All other substances used in this study were of the highest quality and analytical grade.

Preparation of Compound

The BTMP was prepared by following a similar procedure as stated in our previous reports.[28] The compound was formed by the reaction of piperidin-4-one hydrochloride with appropriate thiophene carboxaldehyde in a 1:2 ratio in presence of sodium hydroxide, in the ethanol medium. Then it was allowed to cool to room temperature. The solid obtained was recrystallised from 95% ethanol (Scheme 1).



Scheme 1: Synthesis of BTMP.

Evaluation of antioxidant activity

1,1-Diphenyl-2-Picryl Hydroxyl (DPPH$^{\bullet}$) radical scavenging assay

The radical scavenging activity of the BTMP against DPPH$^{\bullet}$ was measured spectrophotometrically in a dark environment using the Brand Williams *et al.* (1995) method.[29] DPPH$^{\bullet}$ is a stable free radical that accepts an electron or hydrogen radical to form a stable diamagnetic molecule. DPPH$^{\bullet}$ is decreased when it combines with an antioxidant that can give hydrogen. The color shift (from deep violet to blue) was recorded. The amount and type of radical scavenger present in the sample determined the intensity of the color generated. 1ml of various concentrations of BTMP was taken, 1ml of DPPH, and 3ml of water were added. BHT was utilised as a reference and the blue color developed was read at 517nm.

$$\text{DPPH scavenging activity (\%)} = (A_0 - A_1)/A_0 \times 100$$

The absorbance of the control is A0, whereas the absorbance of the sample is A1.

2, 2′-Azinobis-(3-Ethylbenzothiazoline-6-Sulfonic Acid) (ABTS$^{\bullet+}$) Assay

The total antioxidant activity of the samples was determined using the Re *et al.* method of the ABTS$^{\bullet+}$ radical cation decolorisation test (1999).[30] As previously mentioned, the percentage of inhibition was computed.

Ferric Reducing Antioxidant Power (FRAP) Assay

Benzie and Strain's FRAP (Ferric reducing antioxidant power) technique was employed to identify the antioxidant potential of BTMP.[31] In the presence of antioxidants, this procedure aims to convert a ferric-tripyridyl triazine complex to its ferrous-colored form.

Nitric Oxide Scavenging Activity

Using the approach of Sreejayan *et al.*, (1997),[32] the activity of the BTMP in scavenging nitric oxide was determined. In brief, 3.0 ml of 10 mM sodium nitroprusside in phosphate-buffered saline was mixed with varying amounts of sample and incubated at 25°C for 150 min. A combination of 0.5 ml of the incubated solution and 0.5 ml of the Griess reagent was used. The chromophore formed by nitrite diazotisation with sulphanilamide followed by coupling with N-1-naphthyl ethylenediamine dihydrochloride exhibited an absorption of 546 nm. As a reference, BHT was utilised. The percentage of inhibition was determined.

Superoxide anion scavenging assay

The superoxide anion scavenging activity was measured using the Liu *et al.*, (1997) method.[33] 1 ml NBT, NADH, and 0.1 ml of BTMP were mixed together. This mixture was incubated for 5 min at 25°C. As a control, the reagent mixture was used, but no sample was added. At 560nm, the absorbance was measured spectrophotometrically. As a reference standard, BHT was used. The inhibition percentage was calculated.

Scavenging Activity against Hydrogen Peroxide

The hydrogen peroxide scavenging ability of the BTMP was evaluated using the Nebavi *et al.*, technique (2009).[34] 2.0 ml of BTMP (5-40µg/ml) and 1.2 ml H_2O_2 in phosphate buffer (pH 7.4). The same procedure was used to make a blank tube without the addition of H_2O_2. After ten minutes of incubation, the absorbance at 230 nm was determined. The reference standard was BHT and the percentage of inhibition was calculated.

Reducing Power Assay

The Oyaizu 1986 method was used to calculate the BTMP's reduction power.[35] Reduced substances react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form a ferric ferrous complex with a maximum absorbance of 700nm. Various concentrations (5-40 µg/ml) of samples were combined with 5 ml of 0.2 M phosphate buffer at pH 6.6. This was mixed with 5 ml of a 1% potassium ferricyanide solution. The mixture was incubated in a water bath at 50°C for 20 min. After cooling, 5 ml of 10% TCA was added, and the mixture was centrifuged for 10 min at 1,000 rpm. The upper layer of the supernatant (5 ml) was mixed with 5 ml of distilled water. This was mixed with 1 ml of ferric chloride (0.1%) and vortexed. The reaction

mixture's absorbance was then determined spectrophotometrically at 700 nm. As a reference, BHT is used.

Metal Ion Chelating Activity

The Dinis *et al.*, 1994 technique was used to calculate the chelation of ferrous ions in the BTMP.[36] 0.05 ml of 2mM Fecl2 was added to the samples. The reaction began with the addition of 5mM ferrozine (0.2ml) to the mixture, which was thoroughly stirred and allowed to stand at room temperature for 10 min. At 562 nm, the absorbance was measured spectrophotometrically, and the Fe^{2+}chelating activity was calculated.

Phosphomolybdenum Assay

The total antioxidant activity of the BTMP was determined using the phosphomolybdenum test.[37] The antioxidants in this test reduce M0 (VI)–M0 (V) and form a green phosphate/molybdate (V) complex at an acidic pH of 0. 3 ml of sample was mixed with 3 ml of reagent containing 0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate and incubated at 95°C for 90 min. The absorbance of the mixture at 695 nm was measured using a methanol blank.

Antimicrobial Activity

Antibiotics and antimicrobials are substances that kill or prevent the growth of bacteria. New compounds that are not based on existing synthetic antimicrobial medications can only be used to avoid antibiotic resistance.[38] The antibacterial properties of BTMP against two bacterial strains, gram-negative *E. coli*, and gram-positive *S. aureus* were investigated in this work. The reference antimicrobial medication for the bacterial species was ampicillin. The control experiment consisted of inoculating pure solvent with microorganisms in a 1:1 ratio onto a plate of solidified agar.

Statistical Analysis

All of the preceding experiments were conducted in triplicate. The outcomes of experimental tests are presented as the mean standard deviation. Using SPSS version 17, the results were analysed using a one-way analysis of variance, and Duncan's multiple range tests were used to compare the group averages.

RESULTS

Effect of BTMP on DPPH Radical Scavenging Activity

The BTMP acts as a free radical scavenger in the same way that BHT does, according to our findings. Although the BTMP's DPPH radical scavenging abilities were slightly lower than those of BHT, it was clear that the BTMP had proton-donating properties, suggesting that they may be used as free radical inhibitors or scavengers. At 5, 10, 20, and 40µg/ml the BTMP inhibited the DPPH radical by 34.45, 37.65, 43.85, and 46.25 percent, respectively. The results of this investigation imply that the radical scavenging capability of BTMP is mediated by their ability to donate hydrogen possibly as primary antioxidants. Figure 1, shows the DPPH radical scavenging activity of the BTMP.

Effect of BTMP on ABTS Radical Scavenging activity

The scavenging properties of the newly synthesised BTMP against $ABTS^{\bullet+}$ were nearly identical to those of regular BHT. Figure 2, shows the ABTS scavenging activity of the BTMP. At a concentration of 40 µg/ml, the percentage of inhibition for BTMP and BHT was 45.23% and 54.26%, respectively.

Effect of BTMP on FRAP Radical Scavenging Activity

The result of the present study revealed that the reducing power of both the BTMP and the BHT is dose-dependent, with concentrations ranging

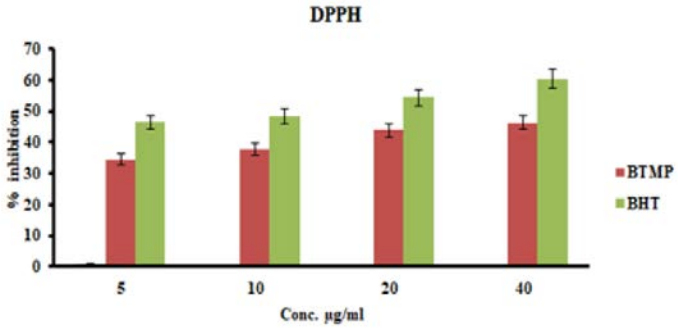


Figure 1: Effect of BTMP on DPPH Assay.

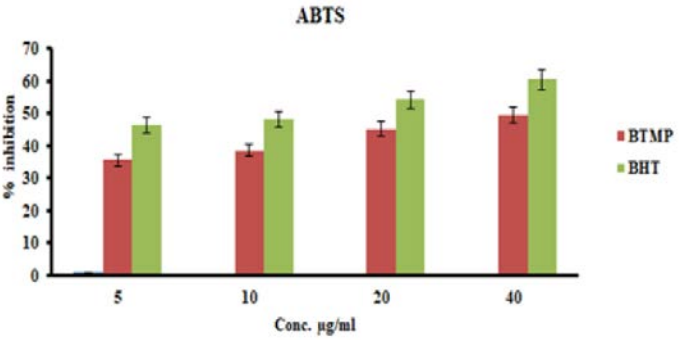


Figure 2: Effect of BTMP on ABTS Assay.

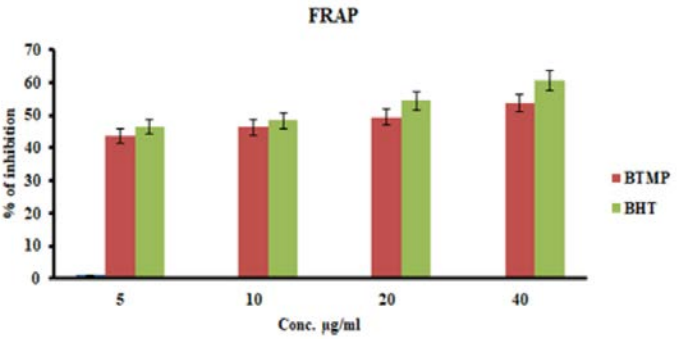


Figure 3: Effect of BTMP on FRAP Assay.

from 5 µg/ml to 40 µg/ml, and their percentage of inhibition is from 42%to 50% and from 44% to 57%, respectively (Figure 3), where the reference compound is near with the BTMP.

Effect of BTMP on Nitric Oxide Scavenging Assay

The percentage-free radical scavenging ability of different concentrations of BTMP is depicted in Figure 4. The NO scavenging capability of the BTMP increased with increased concentration of BTMP, and it had the maximum percentage of inhibition at 40µg/ml is 55%.

Effect of BTMP on Superoxide Anion Radical Scavenging Assay

The BTMP is effective and efficient in preventing the synthesis of blue formazan in a dose-dependent manner, as seen in Figure 5. The proportion of inhibitory concentration increases by the dose from 37% to 47% while going from a low concentration of 5 µg/ml to a higher concentration of 40 µg/ml. The inhibitory concentrations of the reference

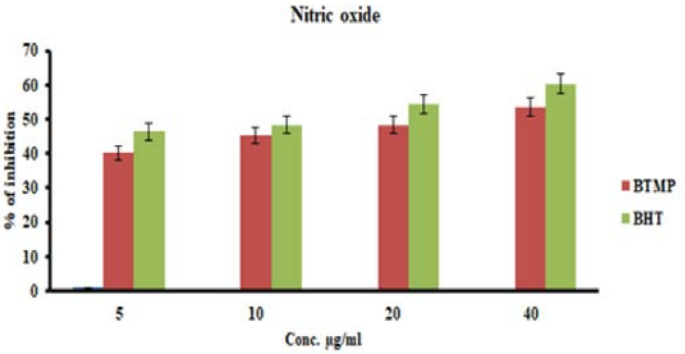


Figure 4: Effect of BTMP on Nitric Oxide.

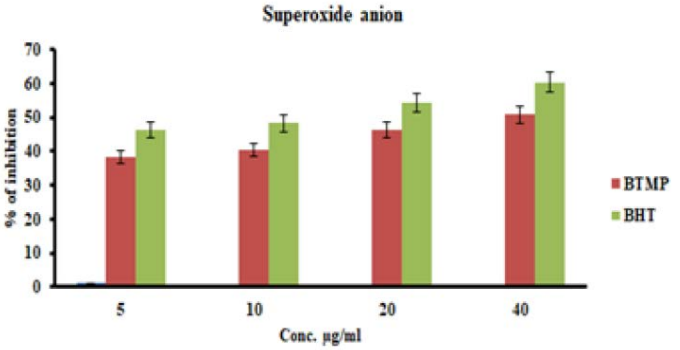


Figure 5: Effect of BTMP on superoxide anion.

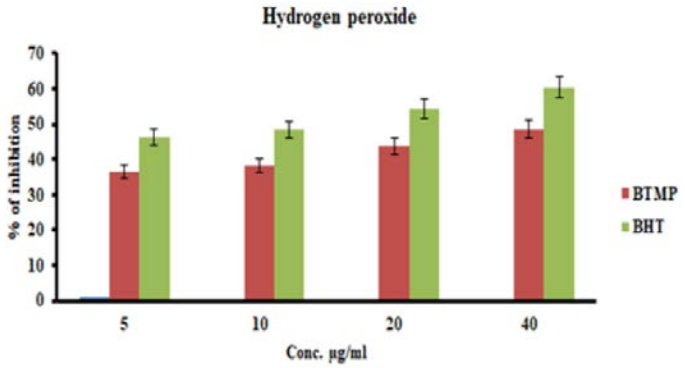


Figure 6: Effect of BTMP on H_2O_2.

substance and the sample derivative are nearly equal, at 40 µg/ml with 45 and 59 %, respectively. These results suggest that BTMP has a potent superoxide radical scavenging effect.

Effect of BTMP on Hydrogen Peroxide Radical Scavenging Assay

Figure 6, reveals that the BTMP has a dose-dependent ability to scavenge H_2O_2 radicals. The BTMP is compared to the reference substance BHT, which has a somewhat higher percentage of inhibition (47-59%) than the sample derivative. The BTMP is noted with a percentage inhibitory concentration of 35 % to 45 % with an increase in the concentration of the dose from 5 µg/ml to 40 µg/ml.

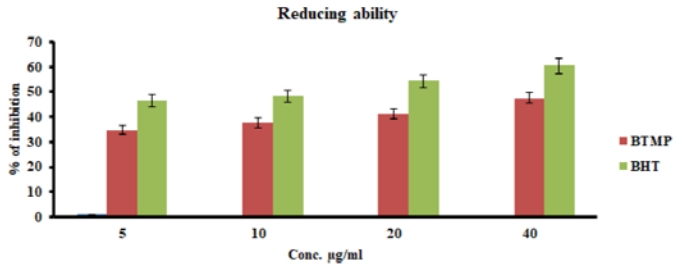


Figure 7: Effect of BTMP on reducing the ability.

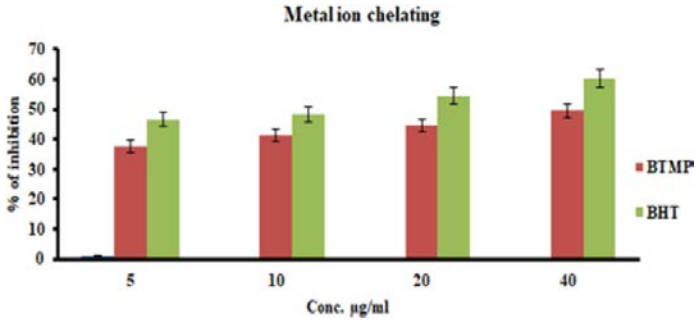


Figure 8: Effect of BTMP on metal ion chelating.

Effect of BTMP on Reducing Power Ability

An increase in the amount of sample and standard concentrations improved the reduction power of the BTMP and the standard with a percentage inhibitory concentration from 35% to 45% and 45% to 60 %, respectively (Figure 7), and the decreasing power of both BTMP and standard exhibits a solid linear connection. The reducing power of BTMP was increased with increasing dosage.

Effect of BTMP on Metal Ion Chelating Ability

The BTMP and standard were tested at concentrations ranging from 5 to 40 µg/ml and revealed substantial chelating activity in a concentration-dependent manner. When the concentration and chelating activity of the sample and the standard are compared, the percentage inhibitory concentration of the sample and the standard is 38% and 45% at 5 µg/ml, respectively, and increases to 50% and 61% at 40 µg/ml (Figure 8). The standard, on the other hand, has a higher chelating ability than the BTMP.

Effect of BTMP on Phosphomolybdenum Activity

At a lower concentration of BTMP (5 µg/ml) and the reference molecule BHT a 39% and a 45% inhibitory impact was observed. This could be due to the phenolic nature of the BTMP. The redox properties of phenolic compounds can play an important role in absorbing and neutralising free radicals. Both compounds were found to be effective at reducing molybdate (VI) to molybdate(V). For both the sample and the standard, the highest percentage inhibitory concentration is at 40 µg/ml, with 48% to 58% (Figure 9).

Effect of BTMP on Antimicrobial Activity

This screening result indicates that the BTMP at 40µg/ml was found to be highly active against *E. coli* and *S. aureus* with a diameter of 24 mm and 21 mm respectively (Table 1). The zone of inhibition of standard ampicillin(10mg) against *E. coli* and *S. aureus* was found to be 28 mm and 22 mm respectively (Figure 10, 11). The results of this study confirmed that BTMP acts against *E. coli* and *S. aureus* in a dose-dependent

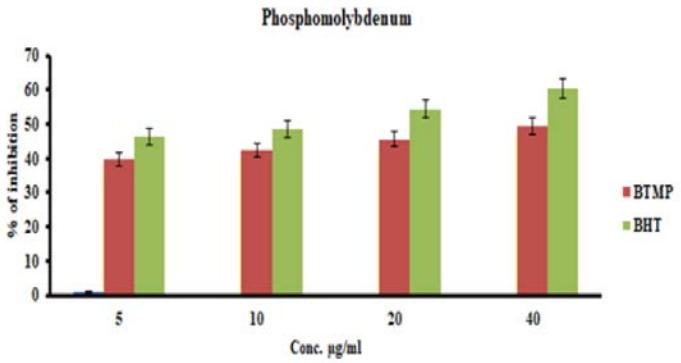


Figure 9: Effect of BTMP on phosphomolybdenum.

Table 1: Antibacterial activity of BTMP against *E. coli* and *S. aureus* (IZ – inhibition zone).

Sl. No	Test microorganisms	Ampicillin (10mg) IZ mm	Zone of inhibition in mm (IZ)			
			BTMP (µg/ml)			
			5	10	20	40
1	*Escherichia coli*	28	19	21	23	24
2	*Staphylococcus aureus*	22	18	19	21	23

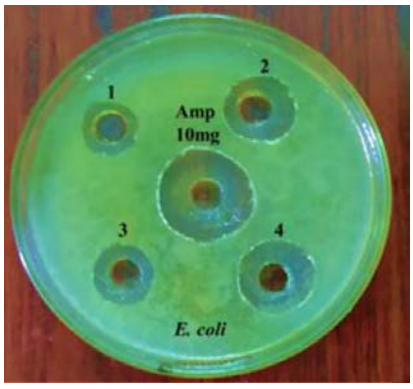


Figure 10: BTMP on antibacterial activity of *E. coli*.
BTMP (5 µg/ml), 2. BTMP (10 µg/ml), 3. BTMP (20 µg/ml), 4. BTMP (40 µg/ml) AMP; Ampicillin standard(10mg)

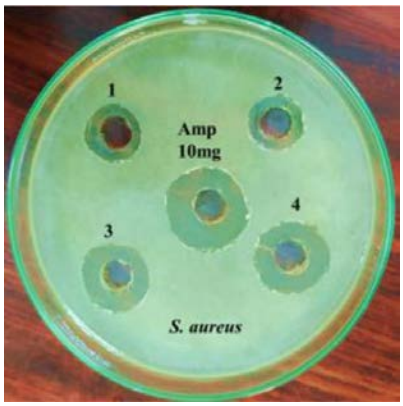


Figure 11: BTMP on antibacterial activity of *S. aureus*.
1. BTMP (5 µg/ml), 2. BTMP (10 µg/ml), 3. BTMP (20 µg/ml), 4. BTMP (40 µg/ml) AMP; Ampicillin standard(10mg)

manner. 40µg/ml of BTMP was found to be more effective in controlling the growth of *E. coli* and *S. aureus*. These experiments confirmed the antibacterial efficacy of the newly synthesised BTMP and suggested the possibility of employing this as a drug for the treatment of infectious diseases caused by the test organisms (*E. coli* and *S. aureus*)

DISCUSSION

Free radicals have a harmful role in many illnesses, including cancer and cardiovascular disease (CVD). The DPPH• free radical scavenging mechanism is a well-known method for testing antioxidant activity. In the DPPH• assay, the BTMP reduces a violet-colored DPPH• solution to a yellow-colored product, diphenyl picryl hydrazine, in a concentration-dependent manner. Because of the short time necessary for analysis, this approach has been widely utilised to predict the antioxidant activity of various compounds. Antioxidants are hypothesised to influence DPPH• because of their propensity to donate hydrogen.[39]

Antioxidant activity can be measured indirectly by using the $ABTS^{\bullet+}$ test. It is known that the ABTS radical is relatively stable in the absence of phenolics; however, the radical undergoes a vigorous reaction with phenolics, an H-atom donor, which results in the formation of an $ABTS^{\bullet+}$.[40] The ABTS assay is used to determine the antioxidants' ability to selectively scavenge $ABTS^{\bullet+}$ and is compared to a BHT. Plant phenolics are recognised to be powerful antioxidants.[41] The phenolic character of the BTMP may be responsible for the observed antioxidant activity. The structure of phenolics, the placement of their hydroxyl atoms, and other features all play a role in their antioxidant and reactive species neutralising abilities.

An antioxidant's ability to reduce a ferric tripyridyl triazine [Fe^{3+}-TPTZ] complex to a ferrous tripyridyl triazine [Fe^{2+}-TPTZ] complex is evaluated using the FRAP assay. Compounds that donate one atom of hydrogen to break the free radical chain are associated with reducing properties.[42] Ferric ion lowering activities of BTMP with reference standard BHT is shown in Figure 3. BTMP's absorbance is increased as the Fe^{2+}-TPTZ combination concentration is increased. As with conventional antioxidants, the BTMP exhibits increased ferric reducing power with increasing concentration.

Nitric oxide is a pleiotropic mediator of several physiological processes. It is a diffusible free radical that acts as an effector molecule in a variety of biological systems.[43] It is a highly unstable species that combines with an oxygen molecule to create stable nitrate and nitrite, which can be quantified using the Griess reagent. The amount of nitrous acid will decrease in the presence of antioxidants, which can be detected at 546 nm.[44] Figure 5 depicts a considerable clearance of NO radical as a result of the scavenging activity of BTMP in a dose-dependent manner. When the percentage of inhibition of BTMP and BHT is compared, the inhibition rate of BTMP is greatly dependent on concentration, and its percentage of inhibition is comparable to that of BHT. The clearance of NO radical can be an indicator of the antioxidant potential of BTMP.

Oxygen-centered radical superoxide is selectively reactive. It is true that superoxide is an extremely inert oxidant with low chemical reactivity, but it can produce more dangerous species like singlet oxygen and hydroxyl radicals, which cause lipid peroxidation.[45] Several enzyme systems are involved in the production of these species. As a result, superoxide anions are precursors to active free radicals, which can react with biological macromolecules and cause tissue damage. The riboflavin/methionine/illuminate system generates superoxide anions from dissolved oxygen, which diminish NBT in this system. The superoxide anion reduces the yellow dye (NBT^{2+}) to create the blue formazan, which is spectrophotometrically detected at 560 nm in this approach. BTMP can prevent the development of blue formazan.

Hydrogen peroxide can be produced by biological systems and it is also generated under physiological conditions from polyphenol-rich beverages.[46] Several oxidising enzymes, including superoxide dismutase, can produce hydrogen peroxide *in vivo*. It can penetrate membranes and may slowly oxidise a variety of substances. H_2O_2 is known to be harmful and to trigger cell death *in vitro*.[47] This radical has the capacity to join nucleotides in DNA and cause strand breaks. Many cellular energy-producing processes can be attacked by hydrogen peroxide. The hydrogen peroxide scavenging ability of the BTMP was evaluated using the Ruch *et al.* techniques.[48] Curcumin is a powerful hydrogen peroxide scavenger.[49] BTMP is a derivative of curcumin, which is also a powerful hydrogen peroxide scavenger as curcumin. Percentage inhibition of hydrogen peroxide by BTMP may be attributed to their phenolic nature, which can donate an electron to the hydrogen peroxide, which neutralises it to water. This removal of hydrogen peroxide by BTMP is very important for life being away from damage.

The radical chain reaction can be stopped when a reducing agent donates electrons to the free radicals, which then causes the radicals to be converted into more stable metabolites. The reduction of potassium hexacyanoferrate (III) is a common method for determining the antioxidant activity and reducing the ability of different compounds.[50] The BTMP's reducing power can be monitored using Perl's Prussian Blue complex production at 700 nm. The antioxidant properties of BTMP may reduce the Fe+3/Hexacyanoferrate (III) complex to Fe^{+2}/ Hexacyanoferrate (III). Reducing power would increase the absorbance of the reaction mixture.

The chelation of ferrous ions by BTMP and standards was determined using the Dinis *et al.* method. Because of its high reactivity, iron is known as the most important lipid oxidation pro-oxidant among the transition metals. Effective ferrous iron chelators may also act against oxidative damage by eliminating ions that would otherwise engage in $OH^{\bullet}$ producing Fenton-type reactions as follows,

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\bullet}$$

The higher concentration of BTMP shows higher antioxidant activity. Ferric ions, like ferrous ions, create radicals from peroxides, but at a 10-fold slower rate.[51] Therefore, BTMP was tested to analyse if it could compete with ferrozine for ferrous ions in the solution.

The total antioxidant potential of BTMP was calculated using their ability to convert molybdate (VI) to molybdate (V), resulting in the formation of a green phosphate/molybdate (V) complex with maximum absorption at 695 nm.[52] The reaction mixture's enhanced absorbance showed an increase in overall antioxidant capacity. All of the varied concentrations in this assay had a strong total antioxidant index, which was concentration-dependent.

Curcumin is the most active component of turmeric that has been explored for its various biological and medicinal properties.[53] In the present study, we focused on the antibacterial activity of newly synthesised BTMP, a curcumin analogue against two genera of bacteria, including those that are Gram-positive (*S. aureus)* and Gram-negative (*E. coli*). Being an amphipathic and lipophilic molecule, BTMP inserts into liposome bilayers and enhances their permeability. Besides, it has also been found that curcumin is involved in disordering the 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) membranes.[54] Hence BTMP has strong antibacterial properties.

CONCLUSION

Based on the results obtained in the present study, it is concluded that newly synthesized BTMP exhibited a wide range of promising antioxidant and antimicrobial activities. BTMP displayed antioxidant activity which was assayed by using different assays including DPPH•, ABTS•$^+$, FRAP, nitric oxide, $O_2\bullet^-$, hydrogen peroxide scavenging, reducing power, metal chelating, and phosphomolybdenum assay. 40µg/ml of BTMP showed the highest antioxidant activity of than other three doses, it is due to its highest free radical scavenging ability at this particular dose. All doses of BTMP showed antimicrobial activity against *E. coli* and *S. aureus*. 40µg/ml of BTMP showed the highest zone of inhibition against both tested pathogens. 40µg/ml of BTMP was considered the effective dosage against the free radicals and pathogens. Further investigations are needed to assess the *in vivo* antioxidant activity of this BTMP prior to clinical use.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

ABBREVIATIONS

ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid); **BHA:** Butylated hydroxy anisole; **BHT:** Butylated hydroxytoluene; **BTMP:** 3,5-Bis(E-thienylmethylene) piperidin-4-one; **CVD:** Cardiovascular disease; **DPPH:** 1,1-Diphenyl-2-picrylhydrazyl; **FRAP:** Ferric reducing ability of plasma; **ROS:** Reactive oxygen species.

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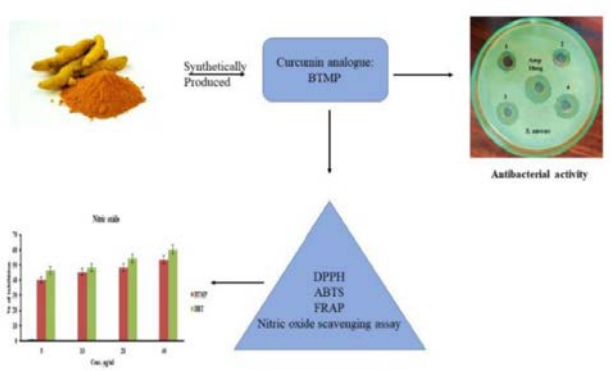
GRAPHICAL ABSTRACT



SUMMARY

- Curcumin is a naturally occurring bis-chalcone derivative present in the commonly used Indian spice turmeric and well-known for its potent chemopreventive, anti-angiogenic, anti-cancer properties.
- BTMP is the synthetic analogue of curcumin and structurally the combination of piperidin-4-ones with arylidene moieties.
- Free radicals have a harmful role in many illnesses, including cancer and cardiovascular disease (CVD).
- BTMP exhibited both antioxidant and antibacterial activity.

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