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In vitro antioxidant and antibacterial activity of Quercetin isolated from Indigofera aspalathoides and Quercetin-Zinc metal complex

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***Abstract*---**Plant secondary metabolites play a crucial role in the therapeutic applications due to their nontoxic nature. The present study was aimed to explore the antioxidant and antibacterial potential of a natural flavonoid Quercetin isolated from the plant Indigofera aspalathoides and Quercetin-Zinc (Qn-Zn) metal complex. The study results explored that, the both Quercetin and Qn-Zn metal complex displayed a strong free radicals quencher against the DPPH, ABTS and superoxide anion free radicals. In addition to that, it has also possessed antibacterial activity against the gram positive (Staphylococcus aureus, Bacillus Subtilis, Enterococcus faecalis) and gram negative (Escherichia coli, Pseudomonas aeruginosa) human pathogenic bacteria. Moreover, we found that and Qn-Zn metal complex displayed slightly better activity than the Quercetin. Overall, the present findings are demonstrated that, the biological activities of natural flavonoid Quercetin were increased, when it's combined with Zinc metal complex.

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***Keywords*---**indigofera aspalathoides, quercetin, zinc metal complex, antioxidant, antibacterial.

Introduction

Medicinal plants are considered as important sources of natural medicine. Since, ancient times herbal plants and plant-based medicine has been used for the treatment of various ailments such as colds, coughs, throat irritations, stomach ache and indigestion gastrointestinal diseases. Still, medicinal plants are broadly used in development of pharmaceutical and nutraceuticals products (Skrovankova *et al.,* 2012). The World Health Organization (WHO) reported that approximately 80% of the public in the world are used traditional medicinal plants for the primary health care. Healthcare experts also commented that the medicinal plants and its products-based treatment is an alternative treatment of various diseases (Alonso-Castro *et al.,* 2017; WHO, 2019).

India is one of the richest plant diversity hot spots in the world. It has been reported that, out of 17,000 species of higher plants identified, 7500 plant species are known for different kinds of medicinal uses. This is highest proportion of using medicinal plants for the medical purposes in any country of the world (Shiva, 1998). In India, Ayurveda is the fundamental source to use of herbal remedies in healthcare. Addition to the Ayurveda system, Siddha and Unani are the other conventional systems of medicine providing more information of plant-based drugs used in India (Raju *et al.,* 2014). People are still using traditional medicinal plants for their primary health care in most of the developing countries including India. It was because, the plant compounds having a broad range of therapeutic applications and low toxic nature. Hence, that may be used in the treatment of major diseases including malaria, cancer and pathogenic microorganisms (Yirga *et al.,* 2011).

The vast range of pharmacological functions of medicines is belonging to the phytochemical constituents. Since, plants phytochemicals are mainly categorised into two types such as primary and secondary metabolites. Plants secondary metabolites are the key molecules for the biological functions of any plants. Further, plant secondary plant metabolites are classified to Phenolics, Alkaloids, Saponins, Terpens, lipids and carbohydrates based on their chemical structures (Hussein and El-Anssary, 2019). Secondary metabolites are reported for wide range of biological applications. It also possesses antibacterial, antifungal and antiviral activity; hence it could able to protect the plants from the pathogens (Bennetts, 1946).

Phenolic compounds are considered as the largest group of plant secondary metabolites. Phenolic molecules particularly flavonoids are widely used in pharmacological applications because of its potential antioxidants and free radical scavenging activity (Goławska *et al.,* 2014). Flavonoids abundantly found in fruits, vegetables, nuts, seeds, stems, flowers, tea, wine, propolis, and honey. Flavonoids are reported for various biological activities including anti-inflammatory, anticancer, anti-allergic and antimicrobial activity (Bone *et al.,* 2012). Flavonoids also have a potential biochemical effect on the cells, which are capable for inhibits enzymes such as, phosphodiesterase, cyclooxygenase, ATPase, lipoxygenase and

aldose reductase. In addition to that, it has been acts as a hormone regulatory molecule by controlling the thyroid hormone, estrogens and androgens (Jain *et al.,* 2019). There are numerous previous studies are reported that, flavonoids exhibited biological activities including antiallergenic, antiviral, anti-inflammatory, and antibacterial activities. Though, those biological activities are due to the antioxidant potential of flavonoids, which has the ability to scavenge the free radical formation (Pietta, 2000).

Recently, antibacterial resistance is one of the important phenomenons of public health worldwide. Overcome this, finding of new antibacterial agents from natural occurring phytochemicals is a potential approach. Moreover, the phytochemicals should have clinically significant importance in order to replace currently available antibacterial agents (Khanam *et al.,* 2015). Flavonoids considered as a one of the most important antibacterial agents against a diverse group of pathogenic bacteria. In the current scenario, increasing incidence of not curable infections induced by antibiotic resistance bacteria, flavonoids have attained much attention because of the antimicrobial potential to be substitutes for antibiotics (Xie *et al.,* 2015).

Quercetin is a kind of polyphenolic compound belongs to the flavonol type class of flavonoids. It was abundantly found in different types of plants, apples, honey, onions, red grapes, citrus fruits and green vegetables (Bathaie, 2015). Quercetin has possessed extensive range of biological actions including antioxidant, analgesic, antiinflammatory, antimicrobial, neuroprotective and antiallergic activity (Ferraz *et al.,* 2021). Due to the vast numbers of biological activities of the quercetin, we have successfully isolated and characterized the quercetin compound from the plant *Indigofera aspalathoides* (Swarnalatha et al. 2015).

Indigofera aspalathoides is belongs to the family of Papilionaceae and commonly called as Sivanar vembu in Tamil. *I. aspalathoides* is a medicinal plant and traditionally used to treat various diseases such as leprosy, syphilis, various skin diseases, inflammation, psoriasis, benign and malignant tumors (Rajkapoor *et al.,* 2004). The plant exhibited various phytoconstituents such as saponins, tannins, steroids, alkaloids, flavonoids, and reducing sugars (Bhuvaneswari, and Balasundaram, 2006). In the previous study it has been reported on antitumor activity against Fibrosarcoma, cervical cancer, Antifungal Activity, anti-inflammatory, anti-viral, antioxidant and hepatoprotective activity (Ramya *et al.,* 2021; Tamilselvi *et al.,* 2011; Sivagnanam *et al.,* 2012; Philips *et al.,* 2010).

Generally, plant bioactive compounds are possessed potent biological activities and widely used in the pharmaceutical industries. However, now a day's researchers are involving to improve the biological activity of plant bioactive compounds through the conjugation of metal such as Zinc, copper and iron. Conjugation of flavonoid compound with metal ions can lead to the generation of novel metallodrugs with enhanced pharmacological functions (Bratu *et al.,* 2014). So, synthesis of compounds analogous from secondary metabolites, such as flavonoids, is a promising source of development of novel drugs. In our previous study, we have reported on the isolation of quercetin compound from the plant *Indigofera aspalathoides* and Quercetin-Zinc complex preparation. Hence, the

study was aimed to identify the antioxidant and antibacterial potential of Quercetin and Quercetin-Zinc (Qn-Zn) complex.

Materials and Methods

In vitro antioxidant Activity

DPPH radical scavenging activity

The DPPH free radical scavenging activity of Quercetin and Qn-Zn complex was assessed by the standard procedure described by Blois (1958). Briefly, 2 mL of different concentration (20, 40, 60, 80 and 100 µg/mL) Quercetin and Q-Zn complex and standard BHT was taken in methanol and mixed with 2 mL of 0.1 mM of DPPH solution. The reaction mixture was incubated for 30 minutes in the dark room and absorbance was measured at 517 nm against a blank containing DPPH in methanol. The DPPH radical scavenging was estimated using the equation.

% Scavenging of DPPH= $[(A_0 - A_1) / A_0] \times 100$

Where, A_0 = absorbance of the control and A_1 = absorbance of the samples

ABTS radical scavenging activity

The ABTS assay of Quercetin and Qn-Zn complex and standard Ascorbic Acid (AA) was carried out by the method described by Arnao et al. (2001). The ABTS working solution was prepared by mixing of 7 mM ABTS solution and 2.4 mM potassium persulfate solution in equal volumes and allowed for 14 hrs in the dark room. Then, fresh ABTS solution was prepared from the stock's solution by diluting 5 times with 0.02 mM of phosphate buffer (pH- 7.0). The reaction was initiated by mixing of 1 mL of different concentration of samples with 1 ml of the ABTS solution and incubated for 7 minutes at room temperature. The absorbance was taken at 734 nm using a spectrophotometer. The ABTS scavenging capacity of the samples were calculated by following formula.

% Scavenging of ABTS = $[(A_0 - A_1)/A_0] \times 100$

Where, A_0 = absorbance of the control and A_1 = absorbance of the samples

Superoxide radical scavenging activity

The superoxide anion scavenging activity was measured as per standard procedure (Robak and Gryglewski, 1998). Superoxide radicals were generated by adding1 ml of NBT (150 µM) and NADH (468 µM) into 3 mL of 100 mM sodium phosphate buffer (pH 7.4). Then different concentrations of samples and standard were added and the reaction was initiated by adding 1 ml of PMS solution (60 µM). The reaction mixture was incubated at 25°C for 5 minutes, and the absorbance was measured against the blank solution. The percentage of superoxide radical scavenging was measured by following formula.

Superoxide radical scavenging activity (%) = $[(A_0 - A_1)/A_0 \times 100]$

Where, A_0 = absorbance of the control and A_1 = absorbance of the samples

Antibacterial activity
Bacterial strains

The antibacterial activity of Quercetin and Qn-Zn complex were evaluated against a human pathogenic bacteria *Staphylococcus aureus*, *Enterococcus faecalism*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis*.

Minimal inhibitory concentration (MIC) assay

MIC of Quercetin and Qn-Zn complex was determined in sterile 96-well plates (Wiegand *et al.,* 2008) and the concentration of the samples is serially diluted from 500 to 1.95µg/mL. Growth and antibiotic control (streptomycin - 15µg/mL) also maintained. The plates were incubated for 24h at 37°C. The resulting turbidity was observed and optical density was measured at 600 nm with UV-Vis Spectrophotometer.

Minimum bactericidal concentration (MBC)

MBC was determination by micro-titre broth dilution method (Ozturk and Ercisli, 2006). It was defined as lowest extract concentration killings 99.9% of the bacterial inoculate post 24 h incubation at 37 °C. 10 microliters were taken from the well obtained from the MIC experiment (MIC value) and two wells above the MIC value well and spread on MHA plates. The bacterial colonies were counted after 24 h of incubation at 37 °C. The concentration of sample that produces <10 colonies was considered as MBC value.

Zone of inhibition

The Quercetin and Qn-Zn complex were dissolved in the sterile double distilled water and used for the assay. Mueller-Hilton agar medium was prepared and poured into sterile Petri dishes followed by addition of 15 ml of seeded medium previously inoculated with bacterial suspension. 6 mm of wells were prepared with the help of sterile cork borer and filled with extracts. Wells loaded with double distilled water used as negative control and streptomycin (15 µg/mL) used as a positive control. The Petri dishes were incubated at 37 ± 1°C for 24 hours. Antibacterial activity was evaluated by measuring the inhibition zone.

Results

DPPH radical scavenging activity

The present study results explored that, the Quercetin and Qn-Zn complex showed promising DPPH radical scavenging activity and we observed a doses dependent increase of radical scavenging activity in both the samples (Fig. 1). The highest percentage (79.98 ± 2.64%) of DPPH radical scavenging activity was noticed at 100 µg/mL of Quercetin and 91.32 ± 2.13 % of inhibition was observed in 100 µg/mL of Qn-Zn complex, whereas the standard sample BHT showed 95.92 ± 1.19 % of inhibition at 100 µg/mL concentration.

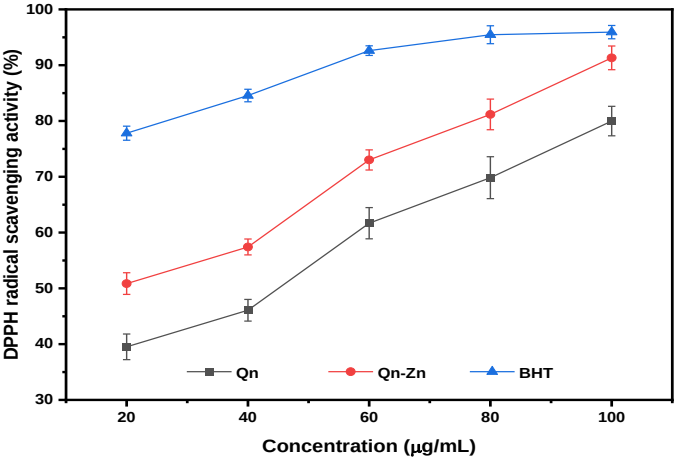

Figure 1. DPPH radical scavenging activity of Quercetin and Qn-Zn complex

ABTS radical scavenging activity

The results illustrated in the Fig. 2 clearly demonstarted that, the Quercetin and Qn-Zn complex has been potentially inhibited the formation of ABTS radicals in the solution. Our resluts explored that, Quercetin showed 84.93 ± 1.17 % of inhibition at the high concentration testsed (100 µg/mL). On the other hand, Qn-Zn complex reached the 86.81 ± 1.76 % of inhibition at the concentration of 60 µg/mL. Here, both the samples showed as a good free radical scavenger compared with standard ascorbic acid showed 95.08 ± 1.29 % of inhibition at 100 µg/mL concentrations.

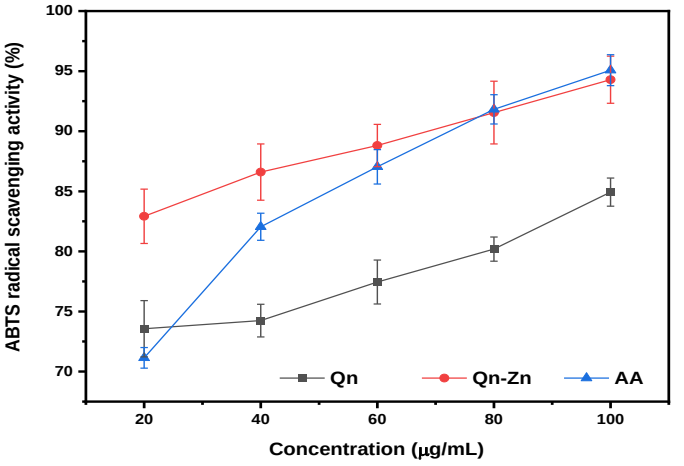

Figure 2. ABTS radical scavenging activity of Quercetin and Qn-Zn complex

Superoxide radical scavenging activity

The trend of superoxide radical scavenging results is similar to DPPH and ABTS radical scavenging activity (Fig. 3). The superoxide radical scavenging activity of

our results showed that 85.6 ± 1.48, 91.95 ± 1.94 and 95.54 ± 1.21% respectively in 100 µg/mL of Quercetin, Qn-Zn complex and Ascorbic Acid.

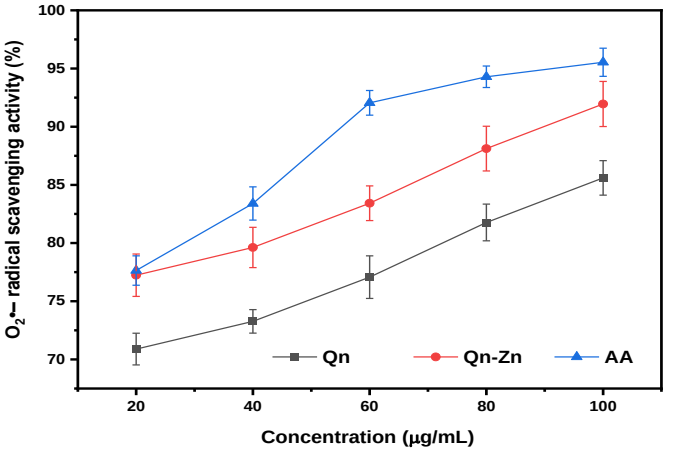

Figure 3. Superoxide radical scavenging activity of Quercetin and Qn-Zn complex

Antibacterial activity

The antibacterial activity of Quercetin and Qn-Zn complex was assessed by various assays such as Minimum inhibitory concentration (MIC), Minimum bactericidal concentration (MBC) and zone of inhibition. The MIC results listed in the Table. 1 clearly showed that, the Quercetin exhibited the MIC of 125 µg/mL against *S. aureus*, *E. faecalis*, *E. coli*, *P. aeruginosa* and 62.5 µg/mL against *B. subtilis*. On the other hand, the Qn-Zn complex showed low MIC than the Quercetin against *B. subtilis* (31.25 µg/mL), *S. aureus* and *E. faecalis* (62.5 µg/mL). Moreover, no variation in MIC was found against *E. coli* and *P. aeruginosa*. Similar to the MIC results, the MBC results also showed Qn-Zn complex exhibited low concentration of MBC than the Quercetin (Table. 2). The zone of inhibition results also supported the MIC and MBC results by the inhibition of bacterial growth (Table. 3). Here also we observed that, the zone of inhibition was found high in the Qn-Zn complex than the Quercetin (Fig. 4). The maximum zone of inhibition 22.29±0.31mm was observed on Qn-Zn complex against *B. subtilis* and the minimum zone of inhibition 11.77±0.29 mm was observed against *E. coli*. Even though, the Quercetin exhibited 18.76±0.41 and 9.18±0.37 mm of zone of inhibition against *B. subtilis* and *E. coli* respectively.

Table 1
Minimum inhibitory concentration (MIC) of Quercetin and Quercetin-Zinc metal complex against various human pathogenic bacterial strains

S. No	Bacterial Strains	Minimum Inhibitory concentration (µg/mL)	
		Quercetin	Quercetin-Zinc metal complex
1	*Staphylococcus aureus*	125	62.5
2	*Bacillus Subtilis*	62.5	31.25

3	*Enterococcus faecalis*	125	62.5
4	*Escherichia coli*	125	125
5	*Pseudomonas aeruginosa*	125	125

Table 2
Minimum Bactericidal concentration (MBC) of Quercetin and Quercetin-Zinc metal complex against various human pathogenic bacterial strains

S.No	Bacterial Strains	Minimum Bactericidal concentration (µg/mL)	
		Quercetin	Quercetin-Zinc metal complex
1	*Staphylococcus aureus*	250	125
2	*Bacillus Subtilis*	125	62.5
3	*Enterococcus faecalis*	250	125
4	*Escherichia coli*	250	250
5	*Pseudomonas aeruginosa*	250	250

Table 3
Zone of inhibition of Quercetin and Quercetin-Zinc metal complex (62.5µg/mL) against various human pathogenic bacterial strains. Data are expressed as mean ± SD (n = 3)

S.No	Bacterial Strains	Zone of inhibition (mm)			
		Quercetin	Qn-Zn Complex	DD H_2O	Streptomycin (15 µg/mL)
1	*Staphylococcus aureus*	11.45±0.43	16.34±0.34	ND	19.56±0.92
2	*Bacillus Subtilis*	18.76±0.41	22.29±0.31	ND	28.50±0.95
3	*Enterococcus faecalis*	9.60±0.38	14.98±0.30	ND	28.04±0.84
4	*Escherichia coli*	9.18±0.37	11.77±0.29	ND	21.50±0.95
5	*Pseudomonas aeruginosa*	12.82±0.32	14.29±0.31	ND	32.83±0.83

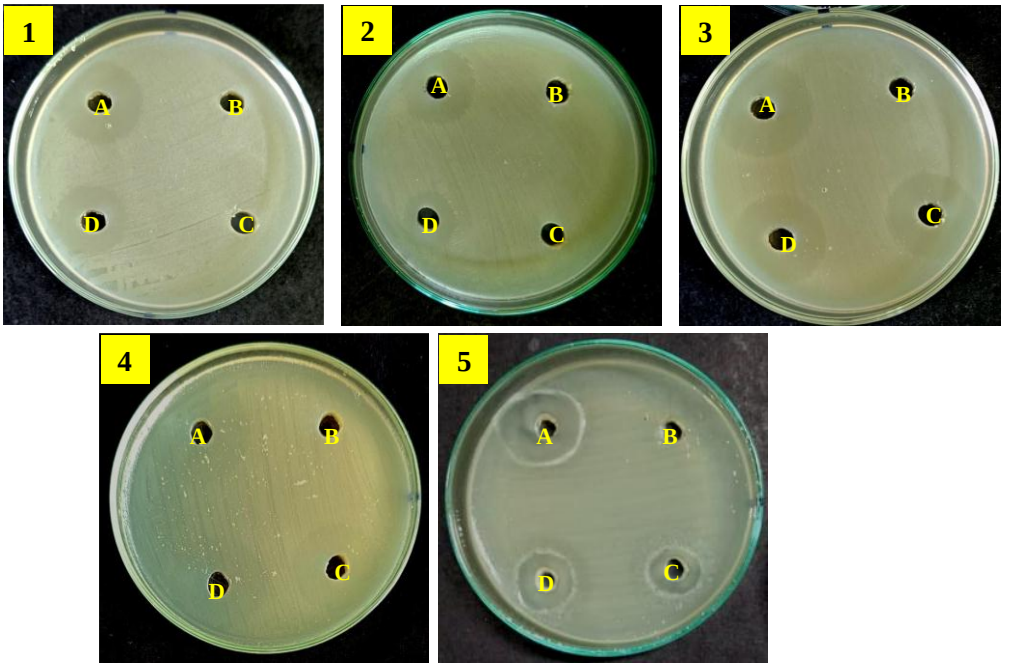

Figure 4. Zone of inhibition of (A) Positive control streptomycin (15 µg/mL), (B) Negative control DD H_2O, (C) Quercetin and (D) Quercetin-Zinc metal complex (62.5µg/mL) against various human pathogenic bacterial strains. (1) *Enterococcus faecalis* (2) *Escherichia coli* (3) *Bacillus subtilis* (4) *Pseudomonas aeruginosa* (5) *Staphylococcus aureus*.

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Discussion

Medicinal plants are a potential source of new chemical substances with promising therapeutic effects against various diseases in the long decades (Wilson and Peter, 1988). Majorly, the antioxidant capacity of the medicinal plant is important to carried out their pharmaceutical applications and contribute to protection against diseases (Eisner, 1990). Moreover, antioxidants from the natural sources are considered as important constituent in the pharmacological industry and which also been much attracted by the scientific community due to the health promoting effect (Saeed *et al.,* 2012). Medicinal plants are well known for the presence of secondary metabolites including phenolic and flavonoids compounds, which has been reported on antioxidant and antibacterial activities because of its redox properties and chemical structures (Safari *et al.,* 2019).

DPPH is a kind of stable free radical and this assay is considered as a crucial assay to find the scavenging activity of any kinds of antioxidants. The antioxidant molecule can reduced the purple color DPPH to Yellow color α, α-diphenyl-β-picrylhydrazine during the neutralization of free stable radical (Suhaj, 2006; Maizura *et al.,* 2011). Our study results explored that, the Quercetin and Qn-Zn metal complex exhibited potential DPPH radical scavenging activity in a dose dependent manner. Previous study on Quercetin and Quercetin Zinc metal complex showed a greater DPPH radical scavenging activity and Quercetin Zinc

metal complex exhibited slightly better activity than the actual compound (Uskokovic-Markovic *et al.,* 2020).

ABTS radical scavenging is actively used to measure both lipophilic and hydrophilic antioxidant. This assay is considered as to be more reliable and accurate, because it has the ability to solubilize in organic and aqueous, stable in wide range of pH (Long *et al.,* 2000). Connection with that, the Quercetin and Qn-Zn metal complex possessed better ABTS radical scavenging activity and explored the strong free radical scavenging activity. Rodriguez-Arce and Saldias (2021) reported that, the flavonoid metal complex showed strong free radicals scavenging activity against ABTS radical's formation.

Superoxide anion radical is one of the most destructive and strongest reactive oxygen species among the other free radicals. It has been directly affecting the bio molecules such as protein and DNA of the cells (Al-Mamun *et al.,* 2007). Hence, the superoxide anion radical scavenging activity is important to protect the tissue from the DNA damage. Our study results findings are explored that both Quercetin and Qn-Zn metal complex greatly inhibit the formation of Superoxide anion radicals. Similar to our study results, a natural flavonoid rutin and Rutin-zinc (II) metal complex exhibited potential antioxidant activity by scavenging the formation of Superoxide anion and DPPH radicals (Ikeda *et al.,* 2015). Addition to the free radical scavenging activity, the Quercetin and Qn-Zn metal complex is also possessed prospective antibacterial activity against both gram positive and Gram-negative pathogenic bacteria. Our study is supported by Uskokovic-Markovic et al. (2020) stated that, the Quercetin-Zinc metal complex overcome a potent antibacterial activity against both gram positive and negative bacterial strains.

In the present investigation, we found that the flavonoid compound Quercetin from the plant *Indigofera aspalathoides* exhibited potential antioxidant and antibacterial activity. However, Quercetin combined with the metal complex Zinc possessed quietly high level of activity than the Quercetin alone. Since, it's has been previously reported that, the flavonoids can form complexes with metal ions and these metal complexes possessed higher free radical scavenging and anti-inflammatory activities and cytoprotective effects than the normal flavonoid compound (Costello and Franklin, 2012). Hence, designing of metal complexes with flavonoid compound is an efficient approach for the development of potential new drugs for the treatment of neurodegenerative diseases (Rodriguez-Arce and Saldias, 2021).

Conclusion

The present study concluded that, the natural flavonoid compound Quercetin isolated from the plant *Indigofera aspalathoides* and Quercetin-Zinc metal complex possessed potential antioxidant activity through the free radical scavenging capacity. In addition to that, it has also showed possible antibacterial activity against human pathogenic bacteria. Further, the Quercetin-Zinc metal complex attained to some extent better activity than the natural compound and explored its importance to the pharmaceutical filed for the development of new drug with metal ion complex.

Conflict of Interest

The authors declare that there is no conflict of interest.

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