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In Vitro Evaluation of Antimicrobial and Antioxidant Activities of *Crossandra infundibuliformis* Root Extracts

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Abstract: Oxidative stress is an important risk factor in the pathogenesis of numerous chronic diseases. The aim of the present study was to evaluate *Crossandra infundibuliformis* (Acanthaceae family) root extract for its antimicrobial, antiinflammatory and antioxidant activities. The root extracts were screened for DPPH (2,2-diphenyl1,1-picrylhydrazyl) radical scavenging activity, Hydrogen radical scavenging activity, Reducing Power activity, Nitric oxide radical Scavenging activity and Hydroxyl radical scavenging activity. The Antibacterial activity of plants extract were determined by Disc diffusion method and Agar well diffusion method. The roots of *Crossandra infundibuliformis* were screened for antibacterial activity against four pathogenic bacteria isolated from clinical samples such as *Bacillus subtilis, Pseudomonas auregonosa, Eischercia coli, Staphylococcus aureus*, by extracting them in ethanol and also anti-oxidant activity using DPPH method. It is also found that *Crossandra infundibuliformis* shows very good anticorrosive property when coated on steel against 1 M HCl. The results of in vitro antioxidant tests suggested that the hydro-alcoholic extract of *Crossandra infundibuliformis* possesses strong free radical scavenging activity that is analogous to a well known standard anti-oxidant ascorbic acid, which could exert beneficial action against pathological alterations caused by the presence of flavonoids.

Keywords: *Crossandra infundibuliformis*, Antimicrobial, Antioxidant, Scavenger, Root extracts, Pathogenic bacteria, DPPH

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Introduction

The antimicrobial agents could be classified as the agents that can either be bactericidal, or bacteriostatic. Antibacterial agents are the most important in fighting infectious diseases. Recently discovered antimicrobial agents are modified natural compounds and this modification is done

through chemical mode, for example, ß-lactams (penicillins), carbapenems, or cephalosporin. Pure natural products, such as aminoglycosides, and other entirely synthetic antibiotics, for example, sulfonamides, are also frequently used. The antimicrobial agents could be classified as the agents that can either be anti-microbial. Antibacterial agents are the most important in fighting infectious diseases. But, with their wide use as well as abuse, the appearance of bacterial resistance toward antibacterial agents has become a major problem for today's pharmaceutical industry (Al-Shehbaz, 2012). Resistance is the commonly taking place for the growth and developmental processes of bacteria, for example, antibiotic therapy, that leads to inheritable resistance.

This increasing resistance of the microorganisms toward antibacterial agents has been responsible in recent years for serious health issues. Most infectious bacteria are resistant (Al-Shehbaz, 2012). This problem motivates the study of new agents that can efficiently inhibit the growth of microorganisms. Nanoparticles have been considered for use in optical devices, fuel cells, catalysts, biosensors, drugs, super-conductors, and gene delivery. Nano-materials as a novel drug delivery vehicle also have been applied to enhance the physicochemical and medicinal effectiveness of drugs. Likewise, nanotechnology in pharmaceuticals and in microbiological study has shown favorable applications to resolve the issue of antibiotic resistance (Sekkoum *et al*., 2015).

An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule. The main characteristic of an antioxidant is its ability to trap free radicals. Oxidative stress is an important risk factor in the pathogenesis of numerous chronic diseases (Sekkoum *et al*., 2011). Free radicals and other reactive oxygen species are recognized as agents involved in the pathogenesis of sicknesses such as asthma, inflammatory arthropathies, diabetes, Parkinson's and Alzheimer's diseases, cancers as well as atherosclerosis (Berreghioua *et al.*, 2014). Reactive oxygen species are also said to be responsible for the human aging. An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule. The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases (Bouchouka *et al.*, 2012). Herbal plants considered as good antioxidant since ancient times.

Crossandra infundibuliformis (Acanthaceae family) is a plant which is important in horticulture. This plant is found abundantly in tropical areas such as South India and Sri Lanka. It reaches 2 m in height and can withstand high temperature which makes it to survive in very high humidity. Due to its medicinal value, the various parts of this plant are used for many treatments. Paliyar tribes of Shenbangathope in Virudhanagar district of Tamil Nadu use flowers of *Crossandra infundibuliformis* in combination with pepper in wound healing (Al Akee *et al*., 2018). The leaf extract of *Crossandra infundibuliformis* shows aphrodisiac activity on ethanol induced testicular toxicity in male rats (El-shabasy, 2016). It is also shown that the increase in aphrodisiac activity is due to the increase in testosterone level. It is also found that *Crossandra infundibuliformis* shows very good anticorrosive property when coated on steel against 1 M HCl (Yusuf *et al*., 2014). Few studies revealed that *Crossandra infundibuliformis* have medicinal values. Despite of studies that have revealed the efficiency of this plant as potential source of aphrodisiac activity, wound healing, anticorrosive activity, etc, there is a lack of information about the level of antibacterial, antioxidant and phytochemical constituents. Therefore, this study investigated antibacterial activity and antioxidant activity through ethanolic root extract of *Crossandra infundibuliformis.*

Materials and Methods

Plant Material collection and extraction:

The roots of the *Crossandra infundibuliformis* were collected from local areas of Visakhapatnam, India. The plant material were identified and authenticated by Botanists, Department of Life Sciences, Andhra University, Andhra Pradesh, India (Christopher, 2008). The material was dried in shade and powdered roots 100 g were extracted with ethanol for 72 h. The extract was dried in the evaporator.

Chemicals:

Ascorbic acid, 2,2 diphenhy-1-picryl hydrazyl hydrate (DPPH), Phosphate buffer, Potassium Ferricynide, Trichloro-acetic acid, were obtained from Sigma Pvt Ltd, India. All the chemicals employed in the study were of analytical grade which were purchased from respective suppliers.

Antioxidant activity:

DPPH (2, 2-diphenyl 1, 1-picrylhydrazyl) radical scavenging activity:

1 ml of of each extract (10-100 µg/ml) or standard was added to 2 ml of DPPH solution in ethanol (0.1 mM). After incubation for 30 min at 37 °C the absorbance of each solution was measured at 517 nm using UV spectrophotometer. Corresponding blanks were taken using ethanol instead of DPPH solution. A similar procedure was repeated with distilled water instead of extract. All analyses were performed in triplicate. The decrease in absorbance indicates increase in free radical scavenging activity. The scavenging activity (%) was measured using the following formula:

$$\% \ SA = \frac{CAbs\text{-}TAbs}{CAbs} \times 100$$

Where SA = Scavenging activity; CAbs= Control absorbance; TAbs= Test absorbance

Antibacterial activity of plants extract:

Disc diffusion method:

The disk diffusion method is used to evaluate antimicrobial activity of the *Crossandra infundibuliformis* extract. The plant extract residues (50 mg) were re- dissolved in 2.5 ml of ethanol, sterilized through Millipore filter (0.22 µm), then loaded over sterile filter paper discs (8 mm in diameter) to obtain final concentration of 10 mg/disc. Ten ml of Mueller-Hilton agar medium was poured into sterile Petri dishes (as a basal layer) followed with 15 ml of seeded medium previously inoculated with bacterial suspension (100 ml of medium/1 ml of 10^7 CFU) to attain 10^5 CFU/ml of medium. Sterile filter paper discs loaded with plant extract concentration of (10 mg/ml) were placed on the top of Mueller-Hilton agar plates. Filter paper discs loaded with 5 µg of Ciprofloxacin was used as positive control. The plates were kept in the fridge at 5 °C for 2 h to permit plant extracts diffusion then incubated at 35 °C for 24 h. The presence of inhibition zones were measured by Vernier caliper and considered as indication for antibacterial activity.

Results

Antioxidant Activity:

The antioxidant activity of root extract has increased gradually from 10 µg/ml to 100 µg/ml and showed promising effect at 100 µg/ml when compared to ascorbic acid as shown in the Table 1 and Figure 1. The H_2O_2 radical scavenging activity of root extract has increased and reached similar activity as compared to ascorbic acid at 100µg/ml as shown in Table 2 and Figure 4. The DPPH activity of root extract has shown similar activity as that of ascorbic acid as shown in Table 4 and Figure 2 and 3. The Nitric oxide scavenging activity of root extract has shown similar activity as that of ascorbic acid as shown in Table 4 and Figure 6. The reducing power root extract has shown similar activity as that of ascorbic acid as shown in Table 3 and Figure 5. Hydroxyl scavenging activity of root extract has also shown promising effect as that of ascorbic acid as shown in Table 5 and Figure 7. All the three activities have proved that extract has good antioxidant

Table 1: DPPH free radical scavenging activity of root extract and ascorbic acid

Concentration (µg/ml)	(%) Scavenging effect of extract	(%) Scavenging effect of extract of Ascorbic acid
10	18.20 ±0.163	33.86 ±0.129
20	22.78 ±0.143	43.59 ±0.209
40	39.55 ±0.418	66.25 ±0.280
60	57.98 ±0.175	75.66 ±0.102
80	72.39 ±0.256	79.32 ±0.193
100	80.11 ±0.100	82.41 ± 0.324

Fig. 1: DPPH free radical scavenging activity of root extract and ascorbic acid.

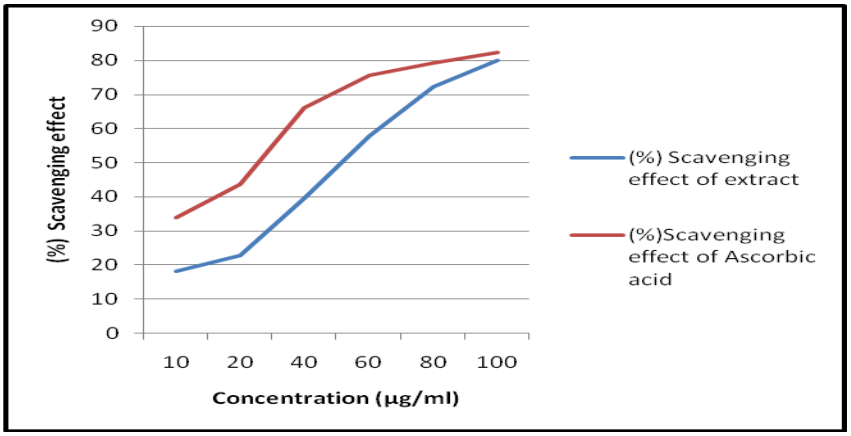

Fig. 2: DPPH free radical scavenging activity of root extract and ascorbic acid.

Fig. 3: DPPH free radical scavenging activity of root extract and ascorbic acid.

Table 2: H_2O_2 radical scavenging activity of root extract and ascorbic acid

Concentration (µg/ml)	(%) Scavenging effect of extract	(%) Scavenging effect of extract of Ascorbic acid
10	33.73 ±0.175	38.69 ±0.217
20	33.61 ±0.220	42.73 ±0.209
40	59.22 ±0.144	63.81±0.150
60	75.35 ±0.178	76.60±0.169
80	78.72 ±0.230	82.97±0.171
100	85.95 ±0.151	89.11±0.221

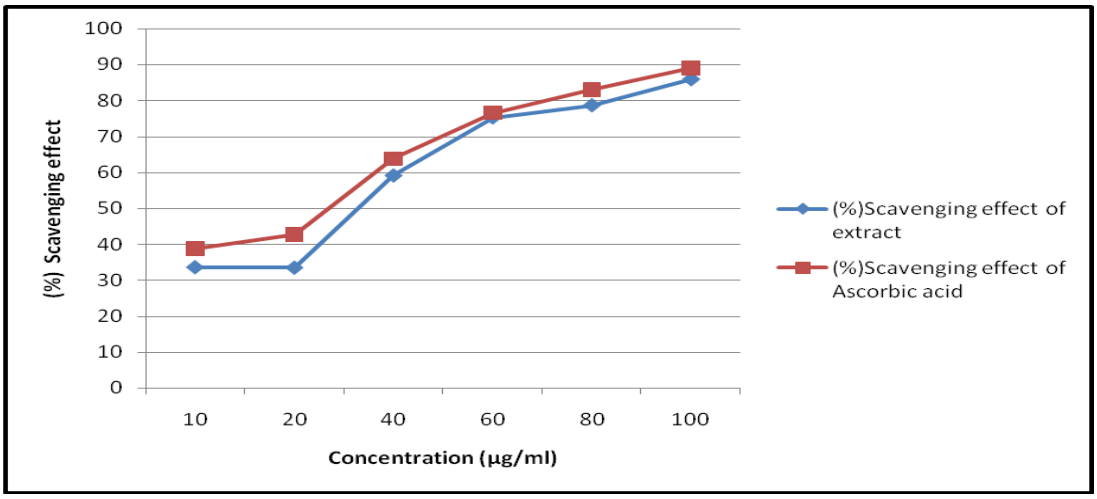

Fig. 4: H_2O_2 radical scavenging activity of root extract and ascorbic acid.

Table 3: Reducing power of root extract and ascorbic acid

Concentration (µg/ml)	Absorbance (extract)	Absorbance (Ascorbic acid)
10	0.310±0.0081	0.427±0.0200
20	0.411±0.0072	0.606±0.0080
40	0.626±0.0065	0.735±0.0065
60	0.670±0.0074	0.789±0.0053
80	0.847±0.0046	0.847±0.0155
100	0.772±0.0036	0.875±0.0102

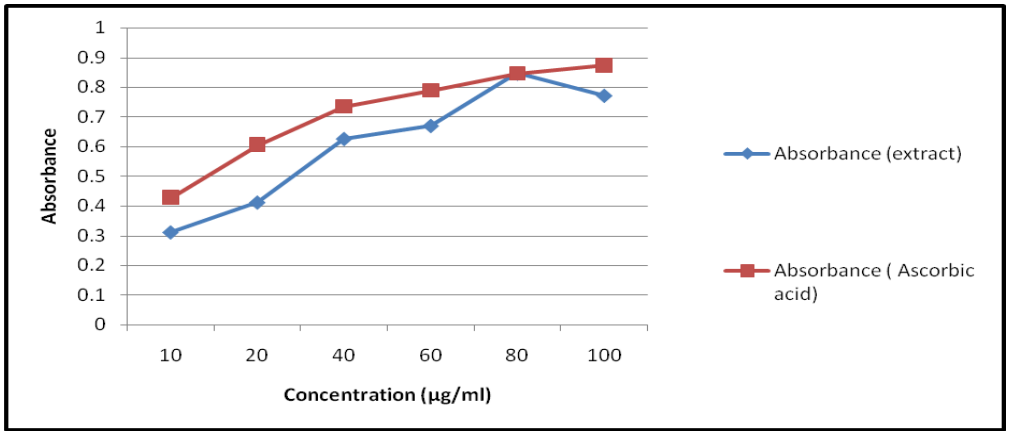

Fig. 5: Reducing power of root extract and ascorbic acid.

Table 4: Nitric oxide scavenging activity of root extract and ascorbic acid

Concentration (ug/ml)	(%) Scavenging effect of extract	(%) Scavenging effect of extract of Ascorbic acid
10	22.11±0.261	25.35±0.082
20	32.72±0.194	35.22±0.157
40	40.24±0.180	40.90±0.181
60	43.62±0.172	48.79±0.407
80	61.30±0.187	61.77±0.212
100	68.88±0.147	71.29±0.307

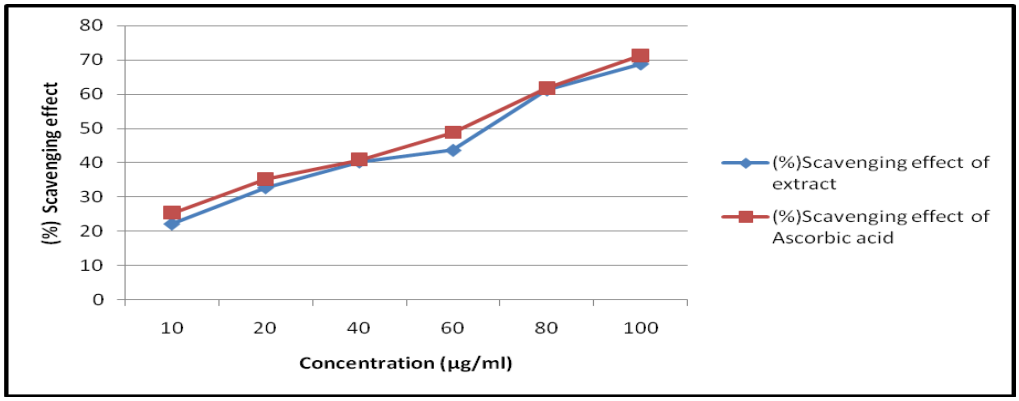

Fig. 6: Nitric oxide scavenging activity of root extract and ascorbic acid.

Table 5: Hydroxyl scavenging activity of root extract and ascorbic acid

Concentration (µg/ml)	(%) Scavenging effect of extract	(%) Scavenging effect of extract of Ascorbic acid
10	15.16±0.137	20.65±0.126
20	22.73±0.162	27.73±0.243
40	42.24±0.214	52.45±0.159
60	53.57±0.196	60.45±0.169
80	60.17±0.169	68.96±0.236
100	71.58±0.145	75.66±0.215

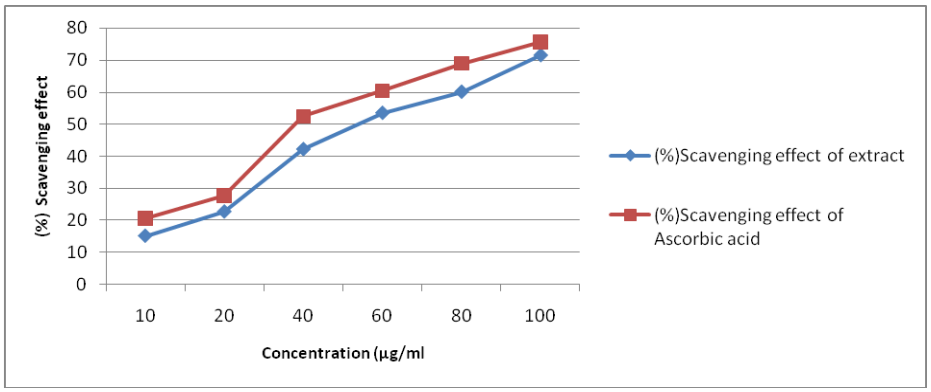

Fig. 7: Hydroxyl scavenging activity of root extract and ascorbic acid.

Table 6: Antibacterial activity of different species

Group	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Staphylococcus aureus*
Standard	25 mm	14 mm	12 mm	13 mm
Control (ethanol)	19 mm	8 mm	6 mm	7 mm
Test 1 (5%)	9 mm	2 mm	1 mm	2 mm
Test 2 (10%)	13 mm	4 mm	3 mm	5 mm
Test 3 (20%)	16 mm	7 mm	5 mm	7 mm
Test 4 (30%)	20 mm	10 mm	7 mm	8 mm

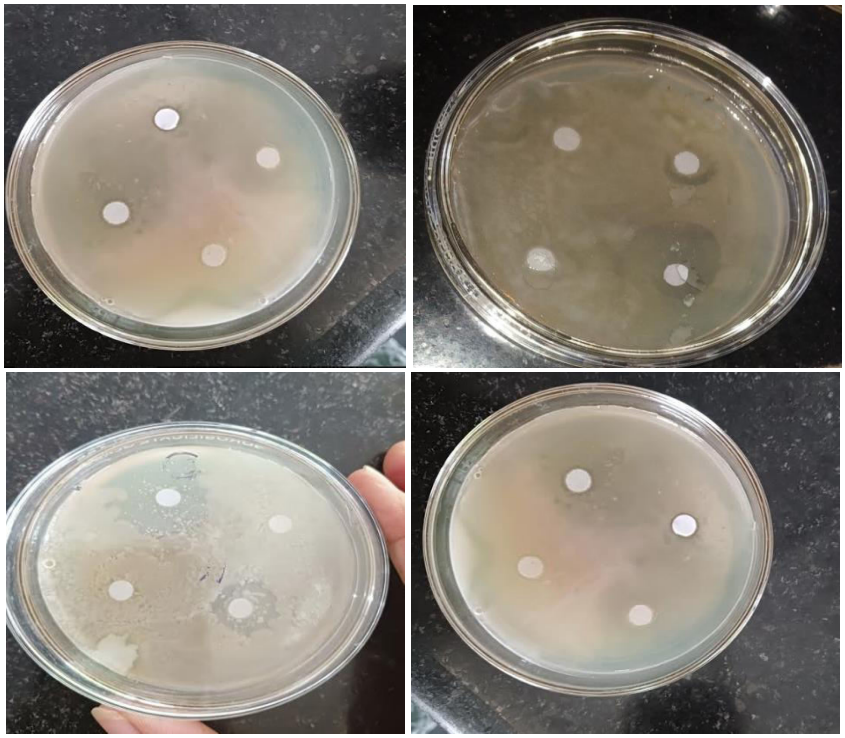

Fig. 8: Antibacterial activity of different species.

activity as compared to ascorbic acid.

Anti Bacterial Activity:

In ethanolic extract the highest antimicrobial activity was at 30% and 20% against all tested microorganisms except *C. albicans* that displayed resistance to all tested concentrations. The lower concentrations (10 and 5%) revealed anti-microbial effect only against gram positive bacteria (*Bacillus subtilis*, *S. aureus*, *S. epidermides* and *B. cereus*) While, gram-negative bacteria (*P. aeruginosa* and *E. coli*) resistant to the lower concentrations as shown in Table 6 and Figure 8.

Discussion

Free radicals are known to play a definite role in a wide variety of pathological manifestations. Their broad range effects in biological systems have drawn the attention of many studies. It has been proven that these mechanisms may be important in the pathogenesis of certain diseases and aging. There are many reports that support the use of antioxidant supplementation in reducing the level of oxidative stress and in slowing or preventing the development of complications associated with diseases (Raghavendra *et al.*, 2019). They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms. DPPH free radical scavenging activity of plant extract and ascorbic acid Proton radical scavenging action is one mechanism for oxidation. The reduction capability of DPPH radicals was determined by the decrease

in its absorbance at 517 nm, suggesting that antioxidant activity of *Crossandra infundibuliformis* extract is due to its proton donating ability and it showed concentration dependent DPPH scavenging activity. *Crossandra infundibuliformis* extract required 2.28 fold the concentration of ascorbic acid to scavenge DPPH (Cowan, 1999).

Antioxidant activity:

Hydrogen peroxide itself is not very particularly reactive with most biologically important molecules, but is an intracellular precursor of hydroxyl radicals which is very toxic to the cell. The decreased absorbance of reaction indicates increased reduction of hydrogen peroxide and hydroxyl radical production (Rios and Recio, 2005). *Crossandra infundibuliformis* root extract scavenged hydrogen peroxide which may be attributed to the presence of phenolic groups that could donate electrons to hydrogen peroxide, thereby neutralizing it into water. *Crossandra infundibuliformis* root extract required a 1.13 fold dose than ascorbic acid to scavenge H_2O_2 (Raj *et al*., 2012).

Reducing power of plant extract and ascorbic acid:

In reducing power assay, the presence of antioxidants in the sample reduced Fe^{3+}/ ferricyanide complex to the ferrous form. This reducing capacity of compounds could serve as an indicator of potential antioxidant properties and increase in absorbance could indicate an increase in reducing power (Radulovic *et al*., 2013). *Crossandra infundibuliformis* root extract required a 2.0 fold dose than ascorbic acid to reach the similar reducing power. Although the greatest reducing power was obtained from ascorbic acid, our results showed that *Crossandra infundibuliformis* root extract is an electron donor and could react with free radicals, convert them to more stable products and terminate radical chain reaction (Compean and Ynalvez, 2014).

Nitric oxide radical scavenging activity of plant extract and ascorbic acid:

Nitric Oxide (NO), a reactive free radical generated from L-Arginine by NO synthase, is well documented as a physiological messenger molecule. Excessive amounts of NO, however, are potentially toxic and have been implicated in numerous pathological situations and chronic inflammation. Scavenger of nitric oxide competes with oxygen leading to reduced production of nitric oxide suggesting that *Crossandra infundibuliformis* root extract showed antioxidant activity. *Crossandra infundibuliformis* root extract required a 1.1 fold dose than ascorbic acid to scavenge nitric oxide radical (Sareedenchai *et al*., 2014).

Hydroxyl radical scavenging activity of plant extract and ascorbic acid:

Hydroxyl radical is the most deleterious and reactive among the Reactive Oxygen System (ROS) and it bears the shortest half-life compared with other free radicals. The oxygen derived hydroxyl radicals along with the added transition metal ion (Fe^{2+}) causes the degradation of deoxyribose into malondialdehyde which produces a pink chromogen with thiobarbituric acid. The *Crossandra infundibuliformis* root extract showed concentration dependent hydroxyl radical scavenging activity and *Crossandra infundibuliformis* root extract required a 1.15 fold dose than ascorbic acid to scavenge hydroxyl radicals (Prashanth *et al*., 2013).

Antimicrobial Activity of ethanol extract:

In ethanolic extract the highest antimicrobial activity was at 30% and 20% against all tested microorganisms except *C. albicans* that displayed resistance to all tested concentrations. The lower concentrations (10 and 5%) revealed antimicrobial effect only against gram positive bacteria (*Bacillus subtilis*, *S. aureus*, *S. epidermides* and *B. cereus*) While, gram-negative bacteria (*P. aeruginosa* and *E. coli*) resistant to the lower concentrations (Badami *et al.*, 2008).This finding is in agreement with previous studies conducted which reported that there was antimicrobial activity against *S. aureus*. Results implied the

alcohol extract of *Crossandra infundibuliformis* roots show high antimicrobial effect against bacterium *Bacillus subtilis* and *Pseudomonas aerugonosa*. As for the activity of ethanol extract of the *Crossandra infundibuliformis* against *Bacillus subtilis* bacterium, the results are in conformity with the previous studies which mentioned that the ethanol extract of the *Crossandra infundibuliformis* roots have inhibitory efficacy against *B subtilis* bacterium. Nishaa *et al.* (2012) reported the antimicrobial activity against *P. aeruginosa* which is in agreement with the present study. Study concluded that the antimicrobial activity of *Crossandra infundibuliformis* root extract on microorganisms increase with the increase in concentration. The activity of some extracts may be due to the presence of compounds such as phytokumanins (a type of phenolic compound that has the ability to break down molecular biofilms). This efficacy was related to the high level of monoterpene hydrocarbons but to the the tannins which bind cell walls of ruminal bacteria. The high activity of the alcoholic extract is due to the low value of acidity (pH) compared to some other extracts. The same studies indicated that *Crossandra infundibuliformis* roots contain several organic acids such as malic, citric, tannic, acetic and other acids that lead to a decrease in the acid function (pH) which increases the antimicrobial efficacy of the extract (Lu *et al*., 2018)

Conclusion

The results of *in vitro* antioxidant tests suggested that the hydro-alcoholic extract of *Crossandra infundibuliformis* possesses strong free radical scavenging activity that is analogous to a well known standard anti-oxidant ascorbic acid, which could exert beneficial action against pathological alterations caused by the presence of flavonids. However, the components responsible for the antioxidative activity are currently unclear. Therefore, further investigations are needed to be carried out to isolate and identify the antioxidant compounds present in the plant extract. Furthermore, the *in vivo* antioxidant activity of this extract needs to be assessed prior to clinical use.

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