

ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF *CYCAS CIRCINALIS* L. AND *IONIDIUM SUFFRUTICOSUM* GING

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ABSTRACT

Objective: To study the antimicrobial and antioxidant activity of *Cycas circinalis* (Cc) and *Ionidium suffruticosum* (Is).

Methods: The ethanolic extract of the above two herbs were taken, and the antimicrobial activity was studied by Kirby-Bauer disk diffusion method and the antioxidant activity was analyzed by percentage inhibition of 1,1-diphenyl-2-picrylhydrazyl activity. Around eight species of microorganisms were taken for antimicrobial assay.

Results: The Cc showed 0.7 mm of the zone of inhibition for *Staphylococcus aureus* in culture, whereas Is showed 1 mm and 0.4 mm of the zone of inhibition for *Escherichia coli* and *Salmonella typhi*, respectively. The rest of all species were nonreactive for both the herbal extracts. The IC₅₀ values of Is at various concentrations were 95.6, 96.0, and 97.6, respectively, whereas, for Cc, it was 78.7, 85.9, and 87.5, respectively. The IC₅₀ value of Is shows that the herb has more antioxidant property when compared to that of Cc and standard (ascorbic acid).

Conclusion: The study shows that Is and Cc both have mild antimicrobial activity acting only on two species of bacteria and the rest were dormant. Is and Cc both were found to have antioxidant activity. This is a preliminary study done on the above two herbs which is a part of vast ongoing research work.

Keywords: Antioxidant activity, Antimicrobial activity, 1,1-diphenyl-2-picrylhydrazyl activity, Kirby-Bauer disk diffusion method, *Ionidium Suffruticosum*, *Cycas circinalis*.

INTRODUCTION

Cycas circinalis. (Cc) L (Family-Cycadaceae) is native of eastern and southeastern Asia and is cultivated in many tropical and subtropical areas for the ornamental purpose [1]. The male sago cone has aphrodisiac activity. Cc is considered to be an invigorating and nutritive tonic for people emaciated by famine or disease. In Siddha system of medicine, the male cone was used to improve maleness. Both male and female cones were having narcotic, stimulant, and aphrodisiac activity [2]. It has been reported that β-N-methylamine, L. alanine from Cc are being implicated in the pathogenesis of human neurological disorders such as lathyrism and amyotrophic lateral sclerosis. Male cones are used as stimulant; seeds are used as aphrodisiac as well as to improve sperm production [3].

Ionidium suffruticosum (Is) (Ging). (Family - Violaceae) is an important medicinal plant in the Indian system of medicine. Traditionally, the herb is used as an aphrodisiac, demulcent, tonic, diuretic, and also used for various ailments such as urinary tract infections, diarrhea, leukorrhea, dysuria, and sterility [4]. This plant is also attributed to its antimicrobial and antiparasitic action. Various phytoconstituents such as dipeptide, alkaloids, aurantiamide acetate, isoarborinol, and β-sitosterol were isolated from this plant [5].

The oxidative stress, defined as the “imbalance between oxidant and antioxidants in favor of the oxidants potentially leading to damage,” has been suggested to be the cause of aging and various diseases in humans. Antioxidants are free radical scavengers prevent pathological conditions of the human body, namely, ischemia, anemia, asthma, arthritis, inflammation, neurodegeneration, and aging process [6]. Many plant extracts and phytochemicals have been shown to have antioxidant or free radical scavenging properties, and it has been established as one of the mechanisms of their actions [7]. Next to antioxidants, the

search for the newer antibiotics is a global challenge, the development of resistance to most of the available synthetic drugs and the high cost of treatment has necessitated the search for alternative antibiotics from natural sources in other way this natural sources will be useful in the development of new, safe, efficient, cost-effective, and ecofriendly manner to deal with pathogenic bacteria's. Hence, a short review of literature was done in search of some herbs having antioxidant and antimicrobial activity, which ended up with two unrevealed herbs that have not been taken for research so far, so an initial trial was done with two herbs Cc and Is, to prove their antioxidant activity and antimicrobial activity.

METHODS

Collection and authentication of plant materials

The Cc dry male cones specimen was identified as per the Siddha literature, authenticated and collected from the trees directly and air dried in shadow for 10 days, and simultaneously, the Is plant was also identified as per Siddha literature, authenticated and collected, washed with distilled water and air dried for 10 days. The dried male cones of cycas and ionidium (whole plant) were powdered using pulverizer and passed through sieve to get fine powder. The 50 g of powder of plant material of Cc and Is were successively extracted with 500 ml of absolute ethanol solvents by hot continuous percolation method in Soxhlet apparatus for 24 hrs. The extracts were concentrated using hot water bath and subjected to drying in a hot air oven [8]. The percentage yield of Cc and Is extracts was found to be 7.14 and 8.42, respectively [9].

Antimicrobial activity (disk-susceptibility tests)

The study was approved by the Saveetha University Animal ethical committee approval reference no- ANAT.005/2012. Disk susceptibilities were determined by the Kirby-Bauer disk diffusion method [10].

Antioxidant activity (1,1-diphenyl-2-picrylhydrazyl [DPPH] free radical scavenging activity)

The antioxidant activity of the plant extracts and the standard (ascorbic acid) were assessed on the basis of the radical scavenging effect of the stable DPPH free radical activity by modified methods [11]. The diluted working solutions of the test extracts were prepared in ethanol. Ascorbic acid was used as standard in 1-100 µg/ml solution. 0.002% of DPPH was prepared in ethanol, and 1 ml of this solution was mixed with 1 ml of sample solution and standard solution separately. These solution mixtures were kept in dark for 30 minutes, and optical density was measured at 517 nm using cecil-electro spectrophotometer. Ethanol (1 ml) with DPPH solution (0.002%, 1 ml) was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below:

Percent (%) inhibition of DPPH activity = $\frac{A-B}{A} \times 100$.

Where, A=Optical density of the blank and B=Optical density of the sample.

All the tests were performed in triplicates and the results were averaged. The inhibition concentration (IC_{50}) was determined. The IC_{50} value was defined as the concentration in mg of dry plant material per ml (mg/ml) that inhibits the formation of DPPH radicals by 50% [11].

RESULTS

The antimicrobial activity of Cc and Is was determined by Kirby-Bauer disk diffusion method with 8 microorganisms, among the eight species, Cc showed the zone of inhibition for *Staphylococcus aureus* (0.7 mm); other microorganisms were nonreactive and dormant, whereas Is showed zone of inhibition for two species *Escherichia coli* (1.0 mm) and *Salmonella typhi* (0.4), whereas other organisms were dormant (Table 1).

The antioxidant activity of Cc and Is was compared to that of a standard using various concentrations of working solution prepared from stock solution, and the percent (%) inhibition of DPPH activity was given in Table 2. The data were analyzed using one-way ANOVA. 10 mg and 20 mg of concentration of standard, Cc, and Is were statistically significant ($p=0.001$), whereas concentration of 30 mg was not significant. Graph 1 show the % free radical scavenging activity. Is has more free radical scavenging activity when compared to standard and Cc at various concentrations.

DISCUSSION

Various compounds isolated from Cc showed moderate antibacterial activity against *S. aureus* and methicillin-resistant *S. aureus*. The isolated biflavonoids were tested for antimicrobial activity. None showed antifungal, antimalarial, or antileishmanial activity [1]. Among the eight species, Cc showed the zone of inhibition for *S. aureus* (0.7 mm); other microorganisms were nonreactive and dormant, whereas Is showed zone of inhibition for two species *Escherichia coli* (1.0mm), *S. typhi* (0.4) other organisms were dormant.

Free radicals can cause oxidative damage to lipids, proteins, and DNA, leading to many chronic diseases and degenerative diseases, such as cancer, diabetes, and aging in humans [12]. Naturally, plants have free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity [13,14]. Various researches have shown that many antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities [15,16]. When these natural antioxidants were consumed through food, it reduces the risks of cancer, cardiovascular disease, diabetes, and other diseases associated with aging [17,18].

Kumar *et al.* carried out a study on the aqueous extract of Is which showed antioxidant activity which is comparable with that of standard drugs.

Table 1: Antimicrobial activity assay in Cc and Is

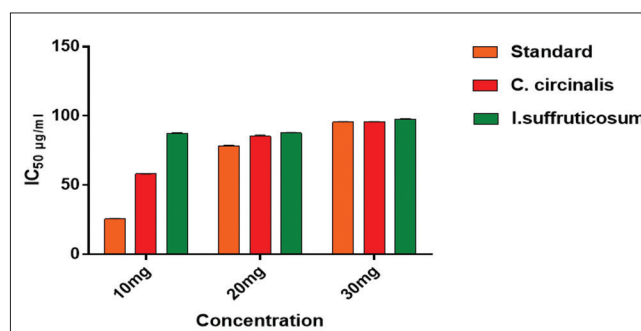
S. No.	Microorganisms	Cc zone of inhibition (mm)	Is zone of inhibition (mm)
1	<i>Aspergillus niger</i>	No zone	No zone
2	<i>Aspergillus flavus</i>	No zone	No zone
3	<i>Aspergillus oryzae</i>	No zone	No zone
4	<i>Penicillium chrysogenum</i>	No zone	No zone
5	<i>Bacillus subtilis</i>	No zone	No zone
6	<i>Escherichia coli</i>	No zone	1.0
7	<i>Salmonella typhi</i>	No zone	0.4
8	<i>Staphylococcus aureus</i>	0.7	No zone

Cc: *Cycas circinalis*, Is: *Ionidium suffruticosum*

Table 2: Antioxidant activity of Cc and Is

Concentration (mg)	IC_{50} µg/ml (mean±SEM)		
	Standard	Cc	Is
10	25.47±0.49	58±0.10	87.36±0.45***
20	78.3±0.56	85.25±0.91	87.5±0.5***
30	95.4±0.47	95.9±0.07	97.7±0.23#

Values are expressed as mean±SEM, #Nonsignificant, Significant, *** $p<0.001$ standard compared to herbal sample solutions, statistical analysis - One-way ANOVA. Cc: *Cycas circinalis*, Is: *Ionidium suffruticosum*



Graph 1: Antioxidant activity of *C. circinalis* L. and *I. suffruticosum* Ging. *C. circinalis*: *Cycas circinalis*, *I. suffruticosum*: *Ionidium suffruticosum*, standard compared to herbal sample solutions, statistical analysis - one-way ANOVA

The aqueous extract of Is was found to have a high content of flavonoids and phenolic compounds. The antioxidant activity of the plant may be due to the presence of the flavonoids and phenolic compounds [5]. In this study, IC_{50} µg/ml was taken. 10 mg and 20 mg of the concentration of standard, Cc, and Is were statistically significant ($p=0.001$), whereas 30 mg of the concentration of standard and herbal extract were not significant (Table 2). Graph 1 shows the antioxidant activity of Cc and Is. As a result, Is has more free radical scavenging activity when compared to that of standard and Cc at various concentrations.

CONCLUSION

Cc and Is were found to be nonreactive against various microorganisms and do not have much antimicrobial activity. The antioxidant activity of both the herbs showed that Is has more free radical scavenging activity when compared to that of Cc. Is was much more effective in restituting the fertility effect in sterility induced male rats when compared to Cc might be possibly due to the presence of flavonoids and various antioxidants as per our previous study. Further, the research will be carried out in future for isolating the specific phytoconstituents responsible for its antioxidant activity.

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