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# EVALUATION OF 2,2-DIPHENYL-1-PICRYLHYDRAZYL, SECONDARY METABOLITE CONTENTS AND ANTIMICROBIAL EFFICACY OF *BLASTANIA GARCINII*(BURM.F.) COGN.

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Conflicts of Interest: Nil
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## ABSTRACT

This study emphasizes on screening of DPPH, secondary metabolites and antimicrobial activity of *Blastania garcinii* an annual vine with slender stem, climbing using tendrils, leaves are ovate and flowers are unisexual. The leaves were collected, shade dried and powdered. The powdered sample was extracted by using soxhlet apparatus with petroleum ether and ethanol. The antioxidant activity of the plant extracts was measured using spectrophotometry by their ability to scavenge free radicals such as DPPH (2,2-diphenyl-1-picrylhydrazyl), Hydrogen peroxide and also quantification of total flavonoid, total phenol content and Antimicrobial activity of plant extracts. The significant result observed in when compared Petroleum ether and Ethanol extract, the DPPH and $H_2O_2$ activity and the total phenol and total flavonoid content and Antibacterial and antifungal activity were showed significant activity in ethanolic extract of *B. garcinii.*

**Keywords:** Antimicrobial activity, DPPH, Flavonoid, Phenol and *Blastania.*

## INTRODUCTION

Medicinal plants have been used to treat human diseases for thousands of years because they have vast and diverse assortment of organic compounds that can produce a definite physiological action on the human body. Most important of such compounds are alkaloids, tannins, flavonoids, terpenoids, saponins and phenolic compounds. Pharmacists are interested in these compounds because of their therapeutic performance and low toxicity[9]. A number of such compounds have been isolated from plants which could be used for the development of new drugs to inhibit the growth of bacterial and fungal pathogens and to quench ROS with possibly novel mechanisms of action and low toxicity to the host cell[1]. In last three decades, a number of new antibiotics have been produced by pharmacological industries but the toxic effects and the global emergence of multi-drug resistant (MDR) of microbes is limiting the effectiveness of these drugs[8]. On account of MDR efflux pump, there is a continuous need to sort out new and innovative therapeutic agents.

Plant phenolics include flavonoids, condensed tannins, coumarins and stilbenes[4]. Phenolics are regarded as the molecules with the highest potential to neutralize free radicals. These compounds act mainly as antioxidants due to their ability to scavenge free radicals and chelate metals *invitro* and *invivo*[15]. Antioxi dants are substances that are able to counter free radical, and they may help to suppress the imbalance that occurs during oxidative stress. They play a key role in protection of plants from pollution damage, disease prevention in both plants and animals, and are very important for the body defenses system[14]. Plants and their secondary metabolites are well known for their antioxidant properties. Antioxidants are micronutrients possessing the potential to either scavenge ROS directly or prevent their generation[5]. Increasing resista nce of microorganisms against available anti microbial agents is of major concern among scientists and clinicians worldwide. In general, it is observed that pathogenic viruses, bacteria, fungi, and protozoa are more and more difficult to treat with the existing drugs[11]. To overcome the drawbacks of the current antimicrobial

drugs and to obtain more efficacious drugs, an antimicrobial drug having a novel mode of action should be developed[11]. Plant-derived flavonoids are a large group of naturally occurring phenylchromones found in fruits, vegetables, tea, and wine. Plants are prospective source of antimicrobial agents in different countries[3]. About 60 to 90% of populations in the developing countries use plant-derived medicine . Traditionally, crude plant extracts are used as herbal medicine for the treatment of human infectious diseases. The main focus of the present study is to determine the DPPH activity, total flavonoid, phenol and antimicrobial activity in leaves of *B. garcinii.*

## MATERIALS AND METHODS

### Collection and Preparation of Plant Extract:

The fresh leaves of *B. garcinii* (Cucurbitaceae) will be collected and shade dried. The plant leaves and25g of powdered *B. garcinii* was successively extracted using 250mL of ethanol and petroleum ether by using the Soxhlet extractor for 8-10 hours [21].

### Determination of Secondary Metabolites:

The total phenolic content of *B.garcinii* ethanol and petroleum ether extract. This test was performed by referring to the method developed by [17].The total flavonoid content of crude extract was determined by [19] method. The total hydrogen peroxide content of the extracts were determined by [20] method.

### Antioxidant Assay

### DPPH Radical Scavenging Activity:

The antioxidant activity of the ethanolic and petroleum ether extraction of *B.garcinii* was measured on the basis of the scavenging activity of the stable 2,2-Diphenoly-1-picryl hydrazyl (DPPH) free radical according to the method described by Brand-Williams[18] with slight modifications. Inhibition % = $A_c$-$A_s/A_c \times 100$.

Where $A_c$ is the absorbance of the control $A_s$ is the absorbance of the sample.

### Antimicrobial Assay

### Antibacterial and Antifungal Assay:

Antibacterial activity of the extracts was determined against three bacterial strains, i.e., *Bacillus subtilis, Klebsiella pneumoniae* and *Salmonella paratyphi* using the well diffusion method. Different concentration of the extracts (50 and 100 µg/ml) was prepared by reconstituting with Ethanol. The antifungal activity against two fungal strains (*Candida albicans* and *Aspergillus fumigatus*) was determined by the well diffusion method. The potato dextrose agar plates were inoculated with each fungal culture (10 days old) by point inoculation. Different concentration of the extracts (50 and 100 µg/ml) was prepared by reconstituting with Ethanol [16].

## RESULTS

### Total Phenolic Content:

The total phenol contents in leaf extracts of *B. garcinii* was analyzed and the results were tabulated in figure 1. Ethanolic extract of *B. garcinii* had higher amounts of total phenolic content (2.451 ± 0.024) than that of petroleum ether extract (0.6615 ± 0.014).

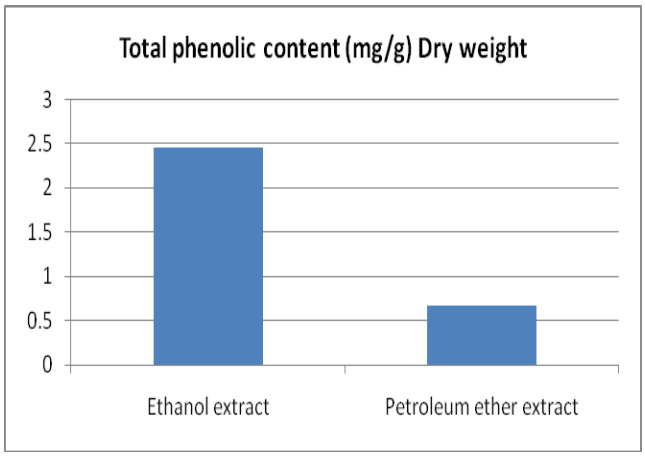


**Figure 1. Total phenolic content in leaf extract of *B. garcinii***

### Total Flavonoid Content:

The total flavonoid content was estimated from the leaves of *B. garcinii* was recorded from the extract of ethanol and petroleum ether. It was found that the ethanolic extract of the leaf

showed high results than that of petroleum ether extract. The ethanolic extract has showed of 0.215 ± 0.005, whereas the petroleum ether showed decreased value of 0.195 ± 0.01 figure 2.

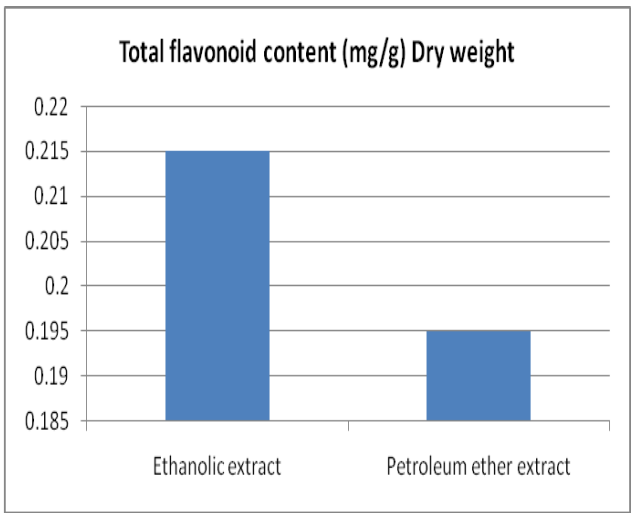


**Figure 2: Total flavonoid content in leaf extract of *B. garcinii***

**Hydrogen peroxide Content ($H_2O_2$):**

The $H_2O_2$ content of *B. garcinii* was significantly decreased in the petroleum ether extract (0.345 ± 0.026), when compare to the ethanolic extract (0.596 ± 0.014). The results are tabulated in figure 3.

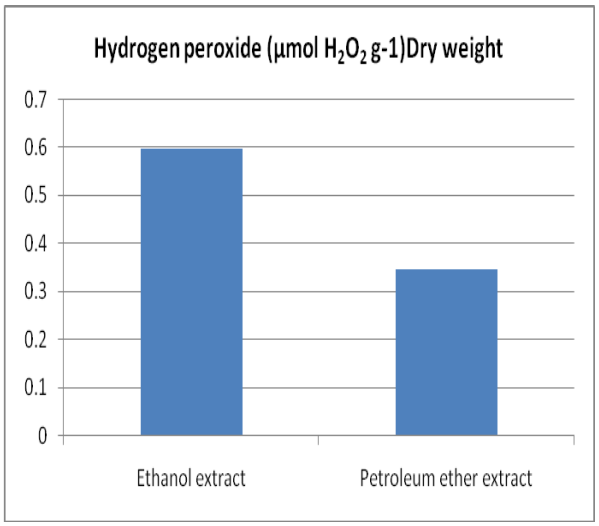


**Figure 3: Hydrogen peroxide content in leaf extract of *B. garcinii***

**Antioxidant Assay**

**DPPH Radical Scavenging Activity:**

The antioxidant activities in leaf of *B. garcinii* ethanol and petroleum ether extracts were assessed by DPPH activity. The DPPH activity of ethanol and petroleum ether extract (100 µg/ml) along with standard ascorbic acid were presented in the table 4 with the positive scavenging activity were noted. The concentra tion (100 µg/ml) of both extracts tested. The higher percentage of inhibition (40.82 ± 0.02) was observed in (100 µg/ml) of ethanol extract followed by (32.04 ± 0.02) 100 µg/ml of petrole um ether extract against the standard ascorbic acid (83.00 ± 0.55). DPPH free radicals have the ability to take electron from the antioxidants that is why it is used for the antioxidants scavenging assays of the medicinal plant for its estimation. figure no.4 shows the percentage scavenging activity in ethanol and petroleum ether leaf extract of *B. garcinii*.

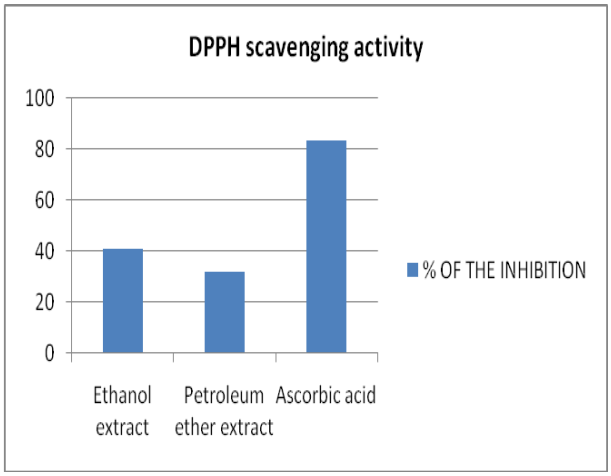


**Figure 4: DPPH scavenging activity in leaf extract of *B. garcinii***

**Antimicrobial Assay**

**Antibacterial Activity:**

In the present study the ethanol extraction of *B. garcinii* exhibited significant antimicrobial activity when compared with standard drug. It is evident from the data presented in the sample possesses antibacterial activity (Figure 5). The disc diffusion method result showed the zone of inhibition for 50µg/ml as 3 ± 0.16mm, 5 ± 0.17mm and 3 ± 0.18mm, for 100µg/ml as 6 ± 0.12mm, 7 ± 0.19mm and 6 ± 0.12, against *B. subtilis, S. paratyphi, K. pneumoniae*. Respecti vely for the test sample when compared with

standard drug Ciprofloxacin showed the zone of inhibition for 9 ± 0.13mm, 11 ± 0.16mm and 11 ± 0.18.

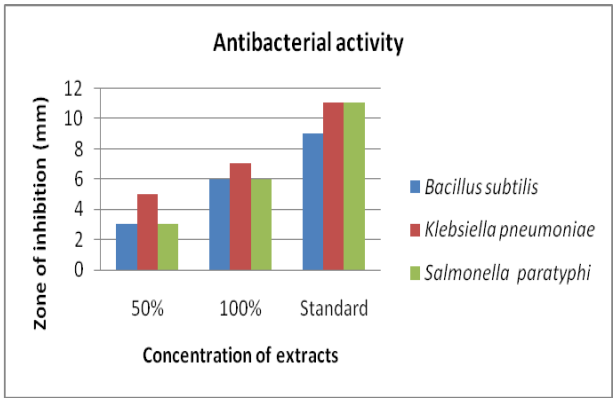


**Figure 5: Antibacterial activity in ethnolic leaf extract of *B. garcinii***

**Antifungal Activity:**

The data presented in that the sample shows antifungal activity (Figure 6). The disc diffusion ethanol result showed the zone 50µg/ml as 2 ± 0.16mm and 2 ± 0.23mm for 100µg/ml as 4 ± 0.25mm and 4 ± 0.16mm against *C. albicans* and *A. fumigatus*. Respectively for the sample when compared with standard drug Ciprofloxacin showed the zone of inhibition 6 ± 0.13mm and 9 ± 0.16. The result showed that the ethanolic extract of *B. garcinii* showed significant antibacterial and antifungal activities against a number of microorganisms.

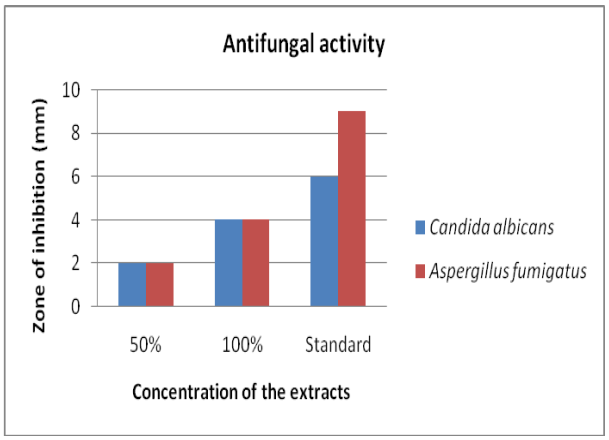


**Figure 6: Antifungal activity in ethanolic leaf extract of *B. garcinii***

**DISCUSSION**

The ethanol and petroleum ether extract of *B. garcinii* showed significant results in Phenol, Flavonoid, Hydrogen peroxide content and DPPH radical scavenging activity. The antioxidant effects on DPPH scavenging was thought to be due to ability of their hydrogen donation capacity[12]. That the ethanolic extract *Lagenaria scieraria* of can be concluded to possess highest amounts of Phenolic, Flavonoid and DPPH free radical scavenging activities. The hydrogen peroxide is a weak oxidizing agent and restrains enzymes by the oxidation of (-SH) groups. Hydrogen peroxide itself is not very reactive but it has the ability to cross cell membrane rapidly and react with $Fe^{2+}$ and $Cu^{2+}$ ions to form hydrogen radical which further leads to toxicity. That the hydrogen peroxide scavenging activity in different extraction of *Coccinia grandis,* the petroleum ether extract showed strong $H_2O_2$ scavenging activity when compare to the other extract. The DPPH radical scavenging of C*eropegia thwaitesii,* ethanol stem and petroleum ether leave extract has 80% of antioxidant activity[13]. It was reported that the five different solvents like methanol, ethanol, petroleum ether, chloroform and aqueous extracts of *Salvinia molesta* leaves showed significant antioxidant activity. Among five different solvent extracts the ethanolic leaf extract has a more effective DPPH radical scavenging activity. There are certain compounds in plants having antibacterial and antifungal activity. The Ethanol extraction of *B. garcinii* shows antibacterial and antifungal activity. Based on the zone of inhibition the antibacterial activity are high when compare to the petroleum ether. The antimicrobial assay of five seeds extract used in study showed the antibacterial activity against both gram positive and gram negative bacteria and against fungal organisms. This antibacterial potency may be due to the presence of many potent compounds such as flavonoids, terpenes, phenolics and alkaloids, it was reported[2]. The same result are observed in ethanolic extraction of *P. granatum* have the highest antibacterial activity against all bacterial isolates than other extracts[7] . Reported that the extraction of ethanolic extracts of *Aframomum citratum* and

*Alchornea cordifolia* prevented the growth of two bacterial strains (*S. aureus* and *S. pyogenes*). Extraction from mature leave and bark of *Avicennia marina, A. officinalis, Bruguiera sexangula, Exoecaria agallocha, Lumnitzera racemosa* and *Rhizophora apiculata* in petroleum ether, chloroform, ethyl acetate, ethanol and water were used to test the growth antibacterial and antifungal activity. The acetate showed the highest inhibition compared to the extracts obtained with petroleum ether, ethanol, chloroform, and water.

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