Evaluation of 2,2-diphenyl-1-picrylhydrazyl, secondary metabolite contents and antimicrobial efficacy of *Blastonia garcinii*(Burm.f.) Cogn.

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Abstract: This study emphasizes screening of DPPH, secondary metabolites and antimicrobial activity of *Blastonia garcinii*an annual wine with a 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 flavonoids, 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 activities and the total phenol and total flavonoïd content and Antibacterial and antifungal activity were shown significant activity in ethanolic extract of *B. garcinii.*

Keywords: Antimicrobial activity, DPPH, flavonoids, phenol and *Blastonia.*

INTRODUCTION

Medicinal plants have been used to treat human diseases for thousands of years because they have a 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, saponin and phenolic compounds. Pharmacists are interested in these compounds because of their therapeutic performance and low toxicity (Inayatullah *et al*., 2012). A number of such compounds have been isolated from plants which could be used

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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 (Ahmad and Aqil, 2007). In the 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 (Hancock, 2005). On account of MDR efflux pump, there is a continuous need to sort out new and innovative therapeutic agents.

Plant phenolic includes flavonoids, condensed tannins, coumarins and stilbenes (Blainski*et al*., 2013). Phenolic 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 *in vitro* and *in vivo* (Sahu and Saxena, 2013).Antioxidants are substances that are able to counter free radicals, and they may help to suppress the imbalance that occurs during oxidative stress. They play a key role in the protection of plants from pollution damage, disease prevention in both plants and animals, and are very important for the body-defense system (Ou *et al*., 2002). 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 (Bursal and Gulcin, 2011).Increasing resistance of microorganisms against available antimicrobial agents are 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 (Koomen *et al*., 2002). 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 (Khalafi-Nezhad *et al*., 2005). 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 (Alviano DS and Alviano CS, 2009). 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 (Alviano DS and Alviano CS, 2009; Zhang *et al*., 2006). 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* were successively extracted using 250 mL of ethanol and petroleum ether by using the Soxhlet extractor for 8-10 hours (Gafner *et al*., 1998).

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 Veliogu *et al*. (1998).The total flavonoïd contents of crude extract was determined by the Bhalodia *et al*., 2011;Patel*et al*., 2010 method. The total hydrogen peroxide contents of the extracts were determined by the Velikova*et al*., (2000) 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 *et al*., 1995) with slight modifications. Inhibition % = A_c-A_s/A_c×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 pneumonia* 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*

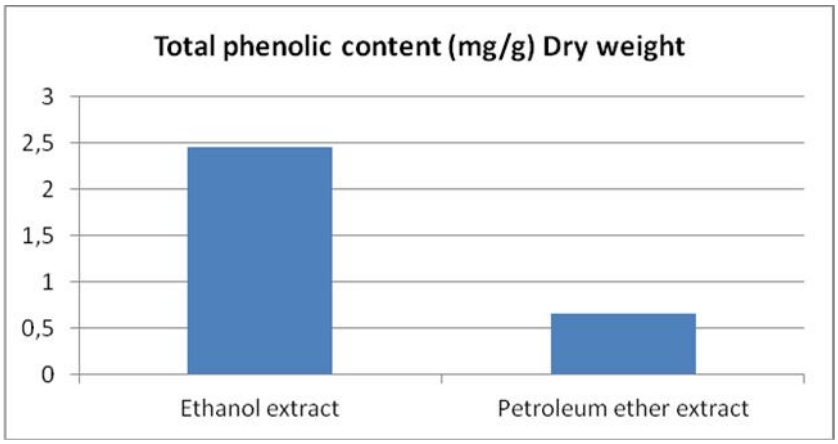
and *Aspergillus fumigatus*) was determined by the well diffusion method. Potato dextrose agar plates were inoculated with each fungal culture (10 days old) by point inoculation. Different concentration of the extracts (50 and 100 µg/ml) was prepared by reconstituting with Ethanol. (Taylor *et al.*, 1995)

RESULTS

Total phenolic content:

The total phenol content 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).

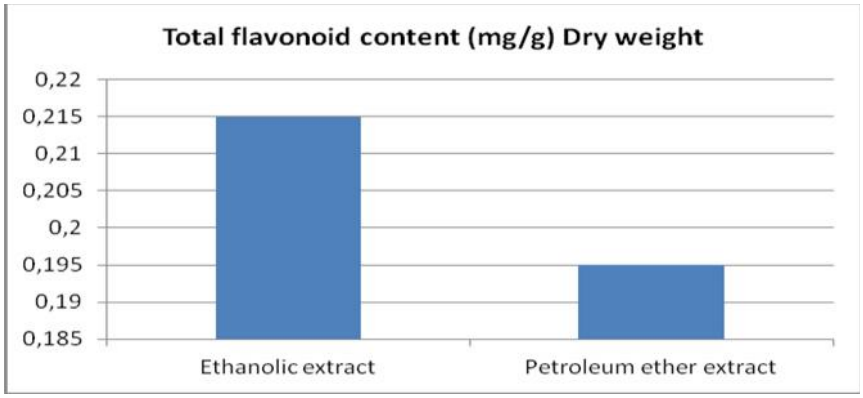
Figure 1. Total phenolic content in leaf extract of *B. garcinii*



Total flavonoïd content:

The total flavonoïd 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 shown 0.215 ± 0.005,whereas the petroleum ether showed decreased value of 0.195 ± 0.01 figure 2.

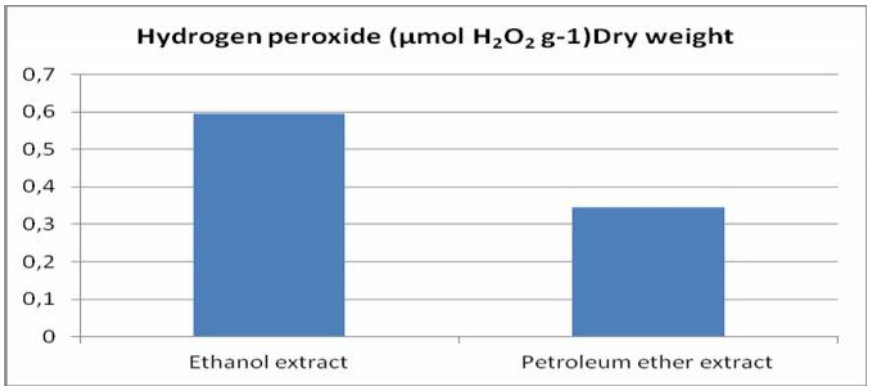
Figure 2. Total flavonoïd content in leaf extract of *B. garcinii*



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 compared 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*

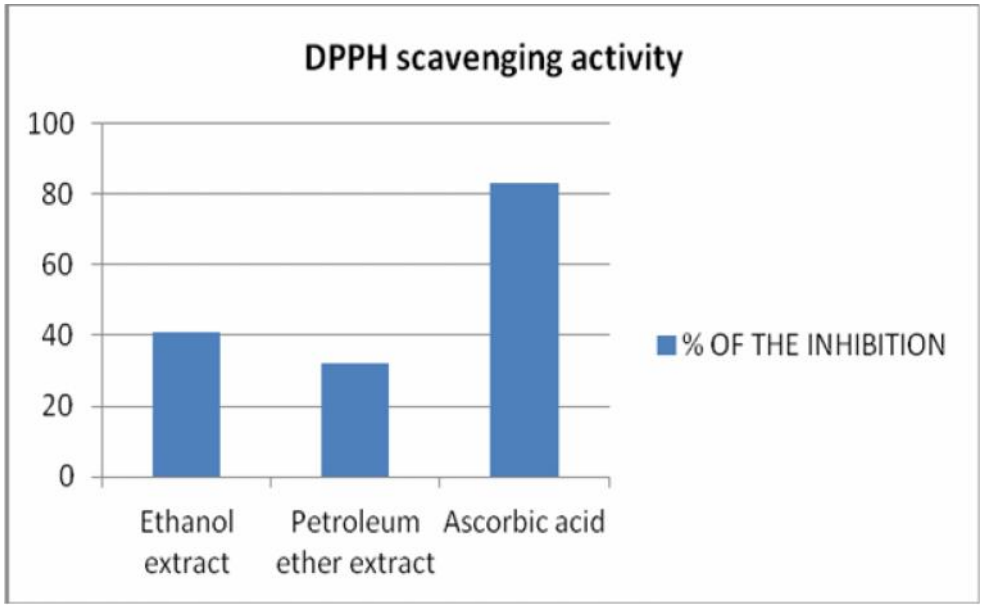


Antioxidant assay:

DPPH Radical scavenging activity:

The antioxidant activities in leaves 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 was presented in the table 4 with the positive scavenging activity was noted. The concentration (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 petroleum ether extract against the standard ascorbic acid (83.00 ± 0.55). DPPH free radicals have the ability to take electrons 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*

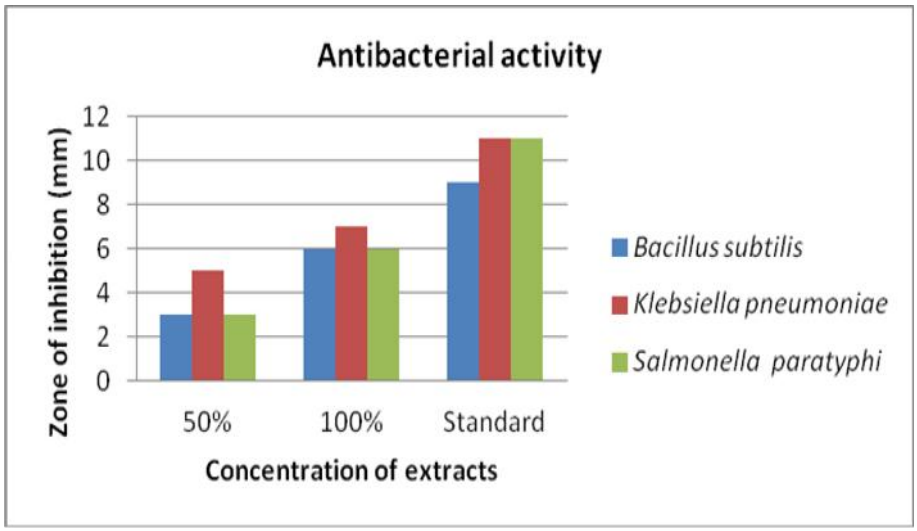


Antimicrobial assay:

Antibacterial activity:

In the present study the ethanol extraction of *B. garcinii* exhibited significant antimicrobial activity when compared with the 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. pneumonia*. Respectively 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 ethanolic leaf extract of *B. garcinii*

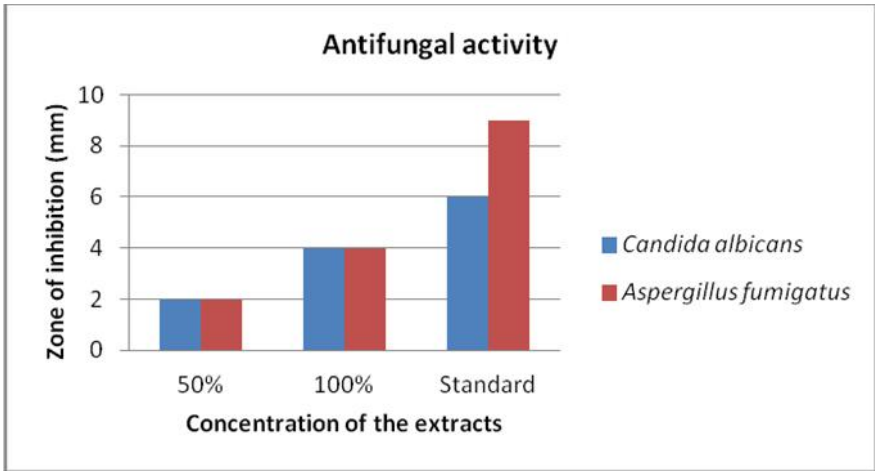


Antifungal activity:

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* shows significant antibacterial and antifungal activities against a number of microorganisms.

Figure No: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, flavonoïd, hydrogen peroxide content and DPPH radical scavenging activity. The antioxidant effect on DPPH scavenging was thought to be due to the ability of their hydrogen donation capacity. Ganesh *et al*. (2019) reported that the ethanolic extract *Lagenaria scieraria* of can be concluded to possess the highest amounts of Phenolic, Flavonoïd 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 the cell membrane rapidly and reacts with Fe^{2+} and Cu^{2+} ions to form hydrogen radicals which further leads to toxicity (Vashishtha VM, 2010). Umamaheswari M and Chatterjee T K, (2008) reported that the hydrogen peroxide scavenging activity in different extraction of *Coccinia grandis,* the petroleum ether extract showed strong H_2O_2 scavenging activity when compared to the other extract. The DPPH radical scavenging of C*eropegia thwaitesii,* ethanol

stem and petroleum ether leave extract has 80% of antioxidant activity. It was reported by Muthukrishnan *et al.,* 2018. Nithya TG, 2016 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. Ethanol extraction of *B. garcinii* shows antibacterial and antifungal activity. Based on the zone of inhibition the antibacterial activity is high when compared 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, phenolic and alkaloids, it was reported by Egwaikhide *et al*. (2010). The same result is observed in (Ali A Alusudani *et al*., 2019) ethanolic extraction of *P. granatum* have the highest antibacterial activity against all bacterial isolates than other extracts. Cousins D & Huffman M. A., (2002) 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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