Phytochemistry and biological activities of the floral hydroethanolic extract of *Ipomoea carnea* Jacq. (Convolvulaceae)

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Abstract

Ipomoea carnea is a species found in Brazil, and is considered toxic to animals. The study aimed to assess the phytochemical composition and biological activities of the floral extract of *I. carnea*. Flowers were collected and the hydroethanolic extract produced. Phytochemistry was evaluated for numerous groups of secondary metabolisms, biological activities were performed to reduce the free radical DPPH, toxicity on *Artemia salina* and photoprotective action. Phytochemical prospecting demonstrated the presence of several classes of phytocomposites of great importance for industries, antioxidant activity with IC_{50} = 13.26 µL mL^{-1}, lethality concentration on *A. salina* with LC_{50} = 455.31µg mL^{-1} and ultraviolet protection factor UVA, UVB and UVC. The hydroethanolic floral extract showed potential with several phytochemical classes and biological activities.

Keywords: *Artemia salina*; Citotoxity; Floral extract; Phytochemical.

Resumo

Ipomoea carnea é uma espécie encontrada no Brasil, considerada tóxica para animais. O estudo teve como objetivo avaliar a composição fitoquímica e as atividades biológicas do extrato floral de *I. carnea*. As flores foram coletadas e o extrato hidroetanólico produzido. A fitoquímica foi avaliada para vários grupos de metabolismos secundários, atividades biológicas foram realizadas para redução do radical livre DPPH, toxicidade sobre Artemia salina e ação fotoprotetora. A prospecção fitoquímica demonstrou a presença de várias classes de fitocompósitos de grande importância para as indústrias, atividade antioxidante com IC50 = 13,26 µL mL^{-1}, concentração de letalidade em A. salina com LC50 = 455,31 µg mL^{-1} e fator de proteção ultravioleta UVA, UVB e UVC. O extrato floral hidroetanólico apresentou potencial com diversas classes fitoquímicas e atividades biológicas.

Palavras-chave: *Artemia salina*; Citotoxidade; Extrato floral; Fitoquímico.

Resumen

Ipomoea carnea es una especie que se encuentra en Brasil y se considera tóxica para los animales. El estudio tuvo como objetivo evaluar la composición fitoquímica y las actividades biológicas del extracto floral de I. carnea. Se recolectaron flores y se produjo el extracto hidroetanólico. Se evaluó la fitoquímica para numerosos grupos de metabolismos secundarios, se realizaron actividades biológicas para reducir el radical libre DPPH, la toxicidad sobre Artemia salina y la acción fotoprotectora. La prospección fitoquímica demostró la presencia de varias clases de fitocompuestos de gran importancia para las industrias, actividad antioxidante con IC50 = 13.26 µL mL^{-1}, concentración de letalidad en A. salina con LC50 = 455.31µg mL^{-1} y factor de protección ultravioleta UVA, UVB y UVC. El extracto floral hidroetanólico mostró potencial con varias clases de fitoquímicos y actividades biológicas.

Palabras clave: *Artemia salina*; Citotoxicidad; Extracto floral; Fitoquímico.

1. Introduction

Convolvulaceae family, is known as morning glory with approx 2,800 taxons belonging to 85 genera from all over the world (Purohit, 2020). *Ipomoea carnea* subsp. *fistulosa* (Fig. 1), a is widely distributed all over the world, as in American tropics, Brazil, Argentina, Bolivia, Pakistan, Srilanka etc (Kunal et al., 2021). In Brazil, and it is popularly known as "algodão-bravo (wild cotton), and mata-cabra (bush-goat)" belongs to the family Convolvulaceae, a species of shrub, woody, little branched, presenting aromatic flowers of pink coloration (Schwarz et al., 2004).

I. carnea is used for its medicinal and ornamental properties. This species has numerous active phytochemical groups, such as alkaloids (indoloozidinic), a potent inhibitor of the lysosomal α-mannose and α-mannose II of the Golgi complex. Other alkaloids such as nortropanics polyhydroxylates are widely distributed in numerous species of the family Convolvulaceae. Calistegines (A, B and C) show inhibitory activity on the β-glycosidase, α-, β-galactosity and β-xylosity enzymes. In addition to alkaloids, studies have described the presence of glycosides and tannins in the leaf organ (Júnior et al., 2020). According by Kunal et al. (2021) the latex of this plant shows anti-inflammatory and antiseptic effects. Water extract of this plant shows anti-rheumatic activity, reduces teratogenic effects of cyclophosphamide, aphrodisiac, purgative, cathartic, sedative and anticonvulsivante activities (Phillips et al., 1994; Ved et al., 2004; Meira et al., 2012). The species is also considered toxic to animals; however, little is known about the floral organ of *I. carnea* and its metabolites and biological activities, requiring studies (Hosomi et al., 2008; Kunal et al., 2021).

The present study was to evaluate the phytochemistry and biological activities of the *Ipomoea carnea* floral hydroethanolic extract.

Figure 1. *Ipomoea carnea* flower and in detail the floral hydroethanolic extract. Source: Authors, 2021

2. Materials and Methods

Plant material

Flowers of *I. carnea* were collected in the county of Rio Verde, Goiás, Brazil. The plant material was identified and a voucher specimen was deposited with the number HRV 15901, at the Herbarium of Vegetable Systematic laboratory, of Goiano Federal Institute, Rio Verde, Goiás, Brazil.

Extract production

Maceration technique using organic solvents (70% EtOH and water) was employed to obtain the floral extract. Powdered sample 150 g were macerated in each solvent 150 mL at room temperature for 7 days. The floral extract was the filtered and concentrated to dryness by using rotary evaporator. The obtained extract was dried (freeze drying) and stored at -12 °C until further analysis described by Cordeiro et al. (2022) modified.

Phytochemical analysis

The phytochemical tests to detect the presence of heterosides, saponins, tannins, flavonoids, steroids and triterpenes, coumarins, quinones, polysaccharides, purines, organic acids, anthraquinones, depsides and depsidones, and alkaloids were performed following the method described by Barbosa et al. (2004) and Menezes Filho et al. (2022). The tests were based on the visual observation of color modification or precipitate formation after the addition of specific reagents.

Antioxidant activity

The method of Mezza et al. (2018) modified was used to evaluate the antioxidant activity in floral extract. The antioxidant capacity analysis was performed by spectrophotometric method of 2,2-diphenyl-1-picrylhydrazyl (DPPH). Ascorbic acid was used as a standard antioxidant. Sample-hexane solution 2 mL prepared at 0.1 and 100 mg mL^{-1} were added to 3 mL of 60 mMol DPPH solution (DPPH 1.2 mg in 50 mL hexane). The mixture was incubated for 120 min, in a dark environment, and absorbance was measured at 517 nm in a spectrophotometer UV-*Vis*. The blank was hexane and the control solution was prepared with 3 mL DPPH solution and 2 mL hexane. The percent (%) of DPPH scavenging effects were calculated. Inhibition concentration (IC_{50}) was defined as the amount of sample (µL mL^{-1}) that produced a 50% in the initial DPPH concentration. Lower IC_{50} values indicate higher free floral extract. The assay was conducted in triplicate.

Brine shrimp lethality assay

The lethality assay in *A. salina* was performed as described by Pereira et al. (2018) adapted. *A. salina* lethality bioassay was carried out to investigate the cytotoxicity of floral extract. *A. salina* were hatched using brine shrimp eggs in a beaker 1L, filled with sterile artificial seawater conc. sea salt 23 g L^{-1} and 0.7 g L^{-1} sodium bicarbonate, and adjusted to pH 8.5 using aqueous solution, conc. 1N of NaOH at room temperature 27 °C and under constant aeration for 48 h. After hatching, active nauplii free were collected and used for the assay. Ten nauplii were drawn through a *Pasteur* pipette and placed in each vial containing 5 mL of brine solution.

In each experiment, 500 µL of the plant extract was added to 5 mL of brine solution and maintained at room temperature for 24 h under the light and surviving larvae were counted. Experiments were conducted along with control (seawater), different concentrations in hydroethanolic solution (35%) (1000; 500; 100; 50; 25 and 1 µg mL^{-1}) of the test substances in a set of four tubes per concentration. The percentage lethality was determined by comparing the mean surviving larvae (*A. salina*) of the test and control tubes. Letal concentration (LC_{50}) values were obtained from the best-fit line plotted concentration verses percentage lethality. Potassium dichromate and seawater was used as a positive control in the bioassay, and negative control containing only 100 µL de DMSO and 5 mL of seawater. The assay was conducted in triplicate.

Photoprotection assay

A scan was performed at the latex between wavelengths 200 to 400 nm in a UV-Vis spectrophotometer, with a 1.0 cm quartz cuvette to verify the absorption in the ultraviolet absorption in the regions (UVA, UVB and UVC) as described by Menezes Filho et al. (2022) and Menezes Filho et al. (2022). The assay was conducted in triplicate.

3. Results

The following results were observed in the floral hydroethanolic extract of I. carnea for phytochemical prospecting (Tab. 1), antioxidant activity and brine shrimp lethality assay (*A. salina*).

Table. 1. Phytochemical prospecting of the main secondary metabolite groups of the floral hydroethanolic extract of *Ipomoea carnea*.

Phytochemical class	Results
Cardiac heterosides	-
Reducing sugars	+
No-reducing sugars	++
Foamy saponins	-
Hemolytic saponnins	++
Tannins	Gr
Flavonoids	+++
Steroids and triterpenes	++
Coumarins	-
Phenols	+++
Polysaccharides	-
Purines	-
Organic acids	+++
Anthraquinones	+
Depsides and depsidones	++
Alkaloids	++

Note: Presence (+++) abundant. (++) moderate. (+) low. (-) absent. (Gr) Green condensed or catechetical tannins. Source: Authors, 2021.

The antioxidant activity of the floral extract of *I. carnea* showed was inhibition concentration IC_{50} = 13.26 ± 0.76 µL mL^{-1}, and acid ascorbic IC_{50} = 7.17 ± 0.06 µL mL^{-1}. The toxicity assay of the floral extract of *I. carnea* with larvae of *A. salina*, the lethal dose capable of killing 50% of the larvae LC_{50} = 455.31 µg mL^{-1}.

The *I. carnea* hydroethanolic floral extract showed bands in the three ultraviolet bands in the respective UVC (265.8 nm), UVB (298.0 nm) and UVA (327.6 nm) waves (Fig. 2).

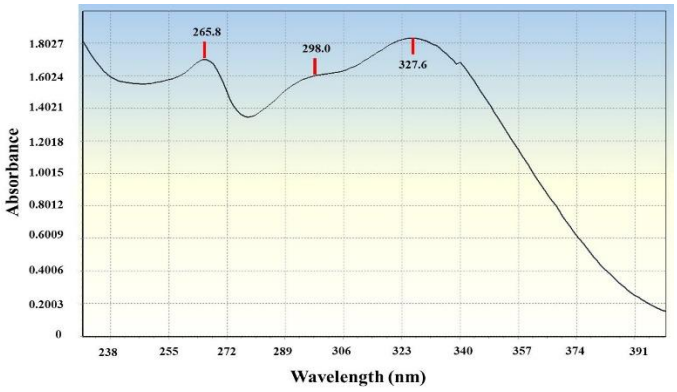

Figure 2. Photoprotection assay by scanning at critical wavelength by UV-Vis spectrophotometry of floral hydroethanolic extract of Ipomoea carnea. Source: Authors, 2021.

4. Discussion

Vegetables within the various botanical families can present numerous active phytochemical classes of great importance for the pharmaceutical, agricultural, biological and biotechnology industries in the development of

formulations with a wide spectrum of use as bactericidal, fungicidal, allelopathic, anticonvulsant, anti-inflammatory, analgesic, molluscicide, insecticide among others. The according to Duarte, Mota and Almeida (2014), and Yadav et al. (2017), the phytochemicals like reducing sugars, no-reducing sugars, alkaloids, flavonoids, tannins, saponins, carbohydrates, purines, cardiac glycosides, phytosterols, phenols, protein and amino acid, diterpens etc. are known to show medicinal activity as well as exhibit physiological actions. The alkaloids have a wide range of pharmacological properties including anti-asthma, antimalarial, antitumor, anticancer properties as reported by Ajuru et al. (2017), and Kittakoop et al. (2014); flavonoids has properties antioxidant, estrogenic, anti-allergic, vascular, anti-inflammatory, anti-carcinogenic, anti-microbial, and anti-viral activity (Sonam et al., 2017).

Coumarins has properties edema modification, faster reabsorption of edematous fluids and treatment of lymphedema (Casley-Smith, 1993); phenols has properties as protecting agents against pathogens, certains type of cancers, neurodegenerative disease, anti-inflammatory, hormone modulators, and diabetes (Ajuru et al., 2017); saponin have been considered as bioactive antibacterial and antifungal agent (Sonam et al., 2017); tannins possess the potential properties as cytotoxic, anti-diarrhoeal and antihemorrhagic agents (Dhivya; Manimegalai, 2013; Sonam et al., 2017); terpenoids are credited for analgesic and anti-inflammatory actions (Sonam et al., 2017); the anthraquinones has properties responsible for the antibacterial and antifungal activity (Oladeji et al., 2020); and purines have anticonvulsants, antileukemic and anti-carcinogenic activities (Řezníčková et al., 2019).

The floral extract showed potential antioxidant activity when compared to the reference ascorbic acid. With this, new studies should be carried out to better evaluate the antioxidant activity of the floral extract of *I. carnea*. According by Mezza et al. (2019) free-radical scavenging activity is mediated by an electron donor molecule, called of antioxidant. Several phytochemical classes such, as flavonoids, tannins and phenolics, have a great reaction preventing chain reaction from oxidation radicals and delaying reactions in the process of degradation (Matthäus, 2002; Orak et al., 2019). The production of reactive oxygen species (e.g. singlete oxygen, and other), occurs physiologically in living organisms, but can also be purchased in the environment. According to Skenderidis et al. (2018), the reactive oxygen species, are useful molecules at low concentrations, since they regulate growth, differentiation, proliferation, and apoptosis cell. Several pathologies are linked to several reactive species such as cardiovascular diseases, neurodegenerative diseases and several types of cancers.

In the studies of Gaur et al. (2009), Abbasi et al. (2010); and Adsul et al. (2012) the researchers reported that the I. carnea methanolic extract was added and dissolved in water, and fractions divided in *n*-hexane, chloroform, alkyl group acetate and *n*-butanol consecutively. The inhibitory effects of these fractions were assessed by DPPH radical inhibition activity, FRAP, and compound total phenolics assay were determined.

In the toxicity assay of the floral extract of *I. carnea* on *A. salina* larvae, the lethal dose capable of killing 50% of the larvae is considered high, according to Meyer et al. (1982). LC_{50} values less than or equal to 1000 µg mL^{-1} indicate potential cytotoxic activity. This relatively fast bioassay makes it possible to know the cytotoxic effect that can be exploited as a possible antitumor and anticancer agent (Saima et al., 2017; Yadav et al., 2020).

The floral extract showed good absorption efficiency in the three wavelengths UVA, UVB and UVC, characterizing the floral extract of *I. carnea* as a possible new natural agent for the production of sunscreens with wide protection. Sunscreens have photoprotective activity, protecting the dermis from the harmful effects caused by different ultraviolet (UV) radiation. Synthetic photoprotective solutions have maximum absorption in different regions of the ultraviolet spectrum. Absorption between 100 to 290 nm corresponds to sunscreens that absorb UVC ultraviolet radiation, between 290 to 320 nm corresponds to UVB sunscreens, and between 320 to 400 nm absorb UVA radiation (Violante et al., 2009). The same ratio is equivalent to photoprotective solutions with incorporated plant extracts that act with the same function to the synthetic, protecting the skin against burns caused by exposure to sunlight.

After the identification of the main constituents and biological activities of *I. carnea* floral extract here observed, we reinforce the importance of researches related of flavonoids, phenolic compounds, tannins, and organic acids biological activities in order to elucidate novel phytotherapeutic approaches for a variety of pathologies and other biological actions. Moreover, we encourage the identification of the possible mechanisms of action of these substances with important pharmacological effects of the (antioxidant, photoprotection and cytotoxic) and medical interest.

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