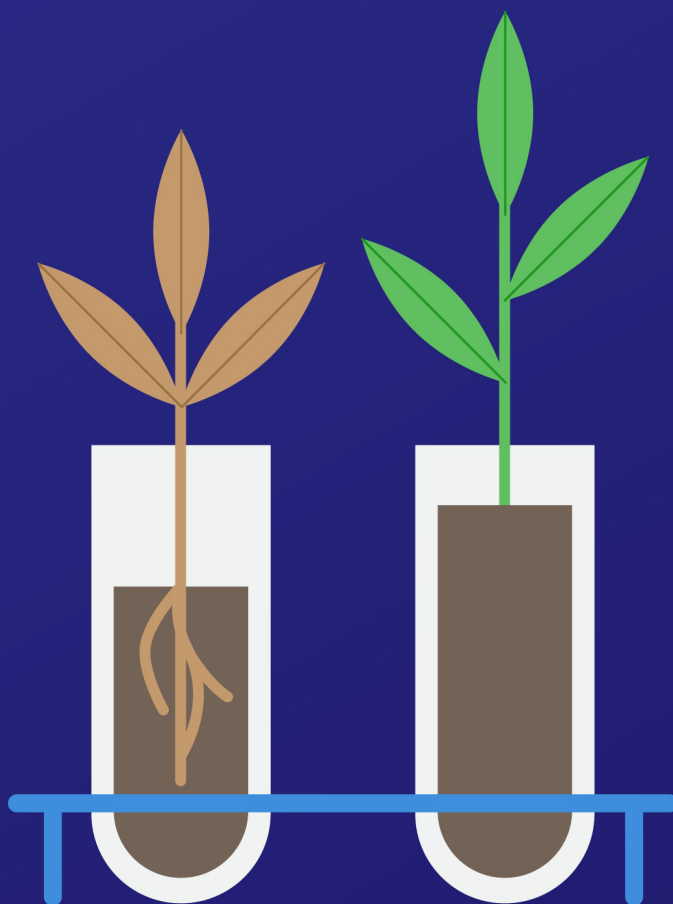




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Phytochemical Analysis on Aqueous Leaf Extract of *Justicia carnea* (Acanthaceae) and its Antibacterial Activity on Some Isolated Bacterial

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ABSTRACT

The present study was aimed to investigate the bioactive constituents and antibacterial efficacy of *Justicia carnea* leaf aqueous extracts on some pathogenic organisms involved in causing infections in humans. The antimicrobial and medicinal properties was examined through phytochemical screening of the plants constituents using standard qualitative methods and conducting bioassay on target bacteria of medical importance such as *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* using the agar well diffusion method. The screening revealed that tannins, saponins and alkaloids is highly detected followed by flavonoids that is moderately detected whereas phlobatannins, phenol and volatile oils are present in low amount. The result shows that the leaf extract was active against *S. aureus*, *K. pneumonia*, *S. typhi*, *E. coli* and *P. aeruginosa* measuring clear zones of inhibition 26.0mm, 22.0mm, 20.0mm, 18.0mm and 18.0mm respectively. Hence, the extracts exhibit strong antibacterial activity and could be used as a preventive and curative measures to common diseases related to the test organisms.

INTRODUCTION

The use of traditional medicine for healing purposes and to treat infections has been practiced since the origin of mankind. It is getting ever more popular in Africa including Nigeria and has been part of African culture in the recent years (Okonkwo, 2012). Serious clinical problems in the treatment of infectious diseases has been created, since microorganisms have developed resistance to many antibiotics. Thus, Plethora of studies has emerged towards using natural products as an alternative therapy (Kumari, *et al.*, 2010). Medicinal plants have been the oldest traditional medicine considered important in treating several human ailments, including bacterial infections (Shyamapada & Manisha, 2011).

Justicia carnea Plant

The genus *Justicia*, named after the 18th-century Scottish

botanist James Justice, belongs to the large family of Acanthaceae consisting of about 600 species of herbs and shrubs native to the tropics and subtropics. *J. carnea* is a flowering plant, widely distributed in various parts of Africa. In Nigeria, the shrubs of *J. carnea* are grown around homesteads and act as fences, which are easy to grow and propagate from stem cuttings by pushing the stems 1 to 2 inches into the soil (Corrêa & Alcântara, 2012).

Taxonomy/Classification of *Justicia carnea*

Kingdom	Plantae
Phylum	Tracheophyta
Class	Magnoliopsida
Order	Lamiales
Family	Acanthaceae
Genus	<i>Justicia</i> L.
Species	<i>Justicia carnea</i> Lindl.
Binomial name	<i>Justicia carnea</i> (GBIF Secretariat, 2021; USDA PLANTS, 2007-2010).

Chemical Composition of Aqueous leaf extract of *Justicia carnea*

Some of the phytochemicals investigated in the leaves include phenols, terpenoids, tannins, alkaloids, carbohydrates, flavonoids, saponins, phenols, reduced sugar and glycosides using standard procedures (Harborne, 1998). Also present in the aqueous leaf extract are vitamin A, B1, B12, B6, B9, B2, C and E containing the highest concentration of vitamin C, and high concentration of iron and calcium while magnesium, zinc and copper were present in low concentrations (Orjiakor *et al.*, 2019).

Hence, the aqueous leaf extract of *Justicia carnea* contains pharmacologically useful active principles elements. Thus



Figure 1: Picture of *Justicia Carnea*

Source: Fieldwork 2023.

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the aqueous leaf extract of the plant could play vital roles in health and disease.

Importance and Uses of *Justicia carnea*

Several species of *Justicia* is one of the many medicinal plants used in Nigeria and it has numerous ethno botanical uses. Traditionally, *Justicia carnea* is used in the management of inflammation, gastrointestinal disorders, respiratory tract infection, fever, pain, diabetes, diarrhea, liver diseases, rheumatism and arthritis (Badami *et al.*, 2003; Corrêa & Alcântara, 2012). They also possess anti-inflammatory, anti-allergic, anti-tumoral, anti-viral and analgesic activities (Radhika *et al.*, 2013).

Justicia carnea is a medicinal plant that is used conventionally as blood tonic from time immemorial in Nigeria. The resultant liquid after boiling is usually crimson red. Local consumers of the leaves are mostly anaemic patients, women who want their blood replenished after the menstrual cycle and pregnant women. It is generally considered as an ornamental plant and it is widely distributed in various parts of Africa. In Nigeria, it is grown around homesteads and acts as fences (Olufunke, 2021).

LITERATURE REVIEW

The preliminary phytochemical analysis of aqueous leaf extract of *J. carnea* revealed that terpenoids, tannins, alkaloids, carbohydrates, flavonoids, saponins, phenols, reduced sugar and glycosides. Phenols and flavonoids were found in high concentrations which could be responsible for the anticancer and antioxidant activities exhibited by the plant (Orjiakor, Uroko, Njoku & Ezeanyika, 2019). The presence of terpenoids and carbohydrates in the aqueous leaf extract of the plant was against the findings of Anigboro, Avwioroko, Ohwokevw, Pessu & Tonukari (2019), who reported their absence in aqueous leaf extract of the same plant. Similarly, Onyeabo, Achi, Ekeleme-Egedigwe, Ebere & Okoro (2017) in their work also reported that phenols, alkaloids, tannins, flavonoids, terpenoids, saponins and steroids were present in the ethanol leaf extract of *J. carnea*, with high percentage of terpenoids, alkaloids and saponins.

According to Igbinađuwa, Kabari & Chikwue (2019) alkaloids, flavonoids, tannins, phenols and saponins were present in the methanol leaf extract of *J. carnea*. In another research, flavonoids, alkaloids, phenols, tannins, carbohydrates, glycosides, gum, protein, fixed oils and fat were reported to be present in methanol leaf extract of *J. carnea*. While alkaloids, fixed oils were absent in the root extract of *J. carnea*, sterols, gum was reported in both stem and root extracts (Sini, Prashy & George, 2018). The absence of saponins in the methanol leaf extract of *J. carnea* was against the report of Igbinađuwa *et al.* (2019) who in their work reported the presence of saponins.

MATERIALS AND METHODS

Study Area

The study was carried out in Federal Polytechnic Bali, Bali

Local Government Area (LGA) of Taraba State, Nigeria (see Fig.2). The Local Government Area lies between latitude 7°46' N and 7°54' N of the equator and longitude 10°30' E and 11°00' E of the prime meridian (Topographic sheet, 1968). This falls within the dry guinea savannah with an estimated land area of 11,540 km². It has some mountains like Gazabu, Dakka, Maihula, Bagoni, among others. Based on the 2006 National Population Census, Bali had a population of about 211,024 persons (NPC, 2006).

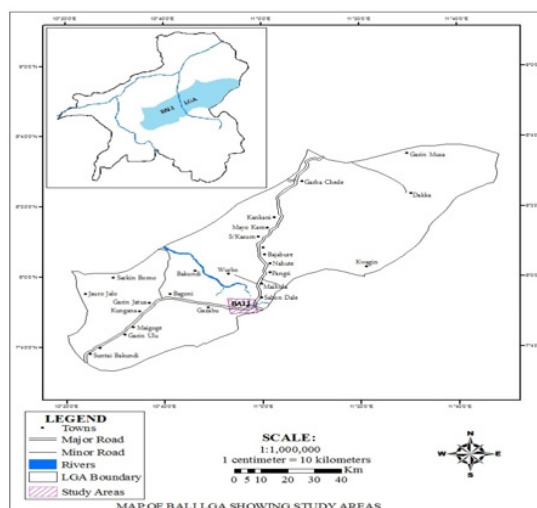


Figure 2: Map of Bali Local Government Area showing the study Area

Source: Bureau for Land and Survey Jalingo, Taraba State.

MATERIALS

Thermostated water bath, Electronic balance (Model: 3003H), Hot plate (Model: ORL 2080), Oven (Thermcool), Desiccators, Cotton wool, Filter paper, Pestle and mortar, Glass wares (volumetric/conical flask, beakers, funnel), *Justicia carnea* leaves

Reagents/ Chemicals

Methanol, Diethyl ether, Ammonia solution, Mayer's reagent, Wagner's reagent, Iron (III) Chloride, Hydrochloric acid, Ethanol, Acetic acid, Tetraoxosulphate (vi) (H₂SO₄) acid, Sodium hydroxide (NaOH)

Preparation of Laboratory Reagents for Phytochemical Analysis

Wagner's Reagent

2g iodine crystals and of 3g potassium of iodide were dissolved in 100cm³ of distilled water and properly stirred to homogenize into a solution.

Ferric Chloride Solution

5g of ferric chloride (Iron III Chloride) was dissolved in 50cm³ of distilled water to form a homogenous solution of ferric chloride.

Dilute Solution of Hydrochloric Acid

2cm³ of hydrochloric acid was measured out using

measuring cylinder and poured into a 250cm³ beaker containing distilled water and stirred to form a homogenous dilute solution of HCl.

Collection and Identification of *Justicia carnea* Leaves

Fresh samples of the leaves of *Justicia carnea* were collected in the month of March, 2023 from Daniya house residence, Bali, Taraba State, Nigeria. The plants was identified using pertinent taxonomic literature (GBIF Secretariat, 2021; USDA PLANTS, 2007-2010).

The plant sample (leaves) were dried on the laboratory table of Science Laboratory Technology Department, Federal Polytechnic Bali, after drying at room temperature the plant was then milled using pestle and mortar and the powder obtained were used for extraction.

Aqueous Extraction Preparation of *Justicia carnea* Leaves

100 gm of fine powdered sample of *Justicia carnea* was weighed and mixed with 500 mL of distilled water in a round bottom flask. A reflux condenser was attached to the flask and inserted with a heating mantle.

The mixture was reflux for one hour and filtered with Watman filter paper No. 1. The reflux was done twice again using new distilled water at each stage. The filtrate was heated to dryness by evaporation. Part of the dry filtrate (extract) was used for phytochemical and antimicrobial screening.

Procedures for Phytochemical Analysis

The phytochemical screening of the plants constituents were assessed by using qualitative methods described by Trease and Evans, 2009.

Alkaloid Test

Equal volumes of the solvent extract (5cm³) and the Wagner's reagent clean test tube and observed for some minutes. The presence of alkaloid was indicated by a brown precipitate.

Phenol

2mL of extract was added to 2mL of ferric chloride solution (FeCl₃), a deep bluish green solution is formed with presence of phenols.

Phlobatannins

1cm³ of hydrochloride acid and 1cm³ of solvent extract were placed into a clean test tube and the tube was heated for about 10 minutes. Reddish green coloration indicates the presence of phlobatannins.

Flavonoids

5cm³ of the solvent extracts was placed into a test tube and few pieces of magnesium chips were added, followed by concentrated hydrochloric acid in drops And then in excess. Reddish colouration indicates the presence of flavonoids.

Saponins (Froth test)

1g of the sample was weighed into a conical flask in 10mL of sterile distilled water was added and boiled for 5 min. The mixture was filtered and 2.5mL of the filtrate was added to 10mL of sterile distilled water in a test tube. The test tube was stopped and shaken vigorously for about 30 second. It was then allowed to stand for half an hour. Honeycomb froth indicated the presence of saponins.

Volatile oils

2.0 mL of extract solution was shaken with 0.1 mL dilute sodium hydroxide and a small quantity of dilute HCl. A white precipitate was formed with volatile oils.

Tannin

3g of the powdered sample was boiled in 50 mL distilled water for 3 minutes on a hot plate. The mixture was filtered and a portion of the filtrate diluted with sterile distilled water in a ratio of 1:4 and 3 drop of 10% ferric chloride solution added. A blue or green colour indicates the presence of tannins.

Microorganisms

The microorganisms used are known to be potentially pathogenic to humans. They include *Escherichia coli*, *Salmonella spp.*, *Staphylococcus aureus*, *Klebsiella spp.* and *Pseudomonas aeruginosa*. All the organisms' strains were obtained as clinical isolates from Sancta Maria Clinic, Bali, Taraba State and Optimum Laboratory, Jimeta-Yola, Adamawa State, Nigeria.

Antimicrobial Susceptibility Testing

The antibacterial activity of *Justicia carnea* leaf aqueous extracts (100mg/ml) in vitro on the isolates was determined by the agar well diffusion method as described by (Olakunle *et al.*, 2013 : Osho & Bello, 2010). This was done using pour plate method in which small colonies from each clinical isolates of the test organisms were made into suspension with 1ml of sterile distilled water in test tubes. 0.1ml of each suspension was dispensed into sterile petri dishes after which melted and sterilized nutrient agar maintained at 45°C was poured (15 aliquot) into the respective plates. The plates were allowed to set, four equidistant wells of 6mm in diameter were punched in each plate using a sterile cork borer. 0.2ml of extract was introduced into each of the wells,. A well filled with sterile water served as control and the plates were allowed to stay for 15 minutes for pre-diffusion to take place followed by incubation for 24-48 hrs at 37°C . The zones of inhibition were measured with the use of a metric rule and were recorded in millimeters.

RESULTS AND DISCUSSION

Table 1 revealed the presence of some chemical/bioactive components in aqueous leaf extract of *Justicia carnea*. Tannins, saponins and alkaloids were highly detected followed by flavonoids that is moderately detected

Table 1: The Result of Chemical Components of Aqueous Leaf Extract of *Justicia carnea*

Phytochemicals	Test	Aqueous Extract
Alkaloids	Wagner's	+++
Tannins	Ferric Chloride	+++
Phlobatannins	Hydrochloric Acid	+
Phenol	Ferric Chloride	+
Flavonoids	Magnesium Chips	++
Saponins	Frothing	+++
Volatile Oils	Sodium Hydroxide	+

+++ = Highly Detected, ++ = Moderately Detected, + = Lowly Detected - = Not Detected

Table 2: Antibacterial activity of Aqueous Leaf Extract of *Justicia carnea* against some bacteria isolates

Organisms (Bacteria)	Dose (ml)	Zone of inhibition (mm)
<i>Staphylococcus aureus</i>	0.2	26
<i>Salmonella typhi</i>	0.2	20
<i>Escherichia coli</i>	0.2	18
<i>Klebsiella pneumonia</i>	0.2	22
<i>Pseudomonas aeruginosa</i>	0.2	18

whereas phlobatannins, phenol and volatile oils are present in low amount. These bioactive compounds have some therapeutic properties against several pathogens supporting its traditional use for curing disease. The presence of these active agents confirms similar research conducted by (Orjiakor *et al.*, 2019). Flavonoids have been acknowledged to possess potent antioxidant and free radical scavenging effect (Ganapath, *et al.*, 2011) and have strong anticancer activity (Del-Rio, *et al.*, 1997; Salah, *et al.*, 1995). They are also documented to have anti-allergic, anti-inflammatory and anti-viral properties and help heal of wounds and treat skin infections due to their ability to neutralize the acidity of wounds and inflammation (Okwu, 2004).

J. carnea extract also revealed to contain saponins. Saponin has the property of precipitating and coagulating red blood cells. Other characteristics of saponins include hemolytic activity, formation of foams in aqueous solutions, cholesterol binding properties and bitterness (Okwu, 2004; Del-Rio, *et al.*, 1997). More so, saponins have also been found to be an antibacterial substance on the cell wall of most organisms (Harbron, 1998). It causes a reduction of blood cholesterol by preventing its re-absorption. The presence of alkaloid indicates that *J. carnea* extract can be used as basic medicinal agents for their antispasmodic, analgesic and bactericidal effects (Okwu & Okwu, 2004; Stray, 1998).

The efficacy of Aqueous Leaf Extract of *J. carnea* against different bacteria is shown in table 2. The sample showed effective antibacterial activity against both gram-positive and gram-negative bacteria as indicated by their inhibition zones. *Staphylococcus aureus* showed greater level of susceptibility (26mm) than other tested bacteria. Followed by *Klebsiella pneumonia* and *Salmonella typhi* with level of susceptibility (22mm and 20mm respectively). *Escherichia coli* and *Pseudomonas aeruginosa* showed the least zone of inhibition (18mm). Hence, *E. coli* and *P. aeruginosa*

were the most resistant isolates tested in this study as they both showed the least zones of inhibition of 18mm.

CONCLUSION

This present study showed that *J. carnea* leaf is a good source of important phytochemical or bioactive components which include tannins, saponins, alkaloids and flavonoids that are involved in both pharmacodynamics, bactericidal and bacteriostatic activity and these compounds are known to have some therapeutic properties against several pathogens which supports its traditional use for curing disease. They are vital sources of supplementary diets of several bioproducts essential for the biochemical functions in human, provide health benefits and are important for prevention illnesses. Therefore, justifies its usefulness in clinical and folklore practices against diseases caused by bacteria. Though, dosage remains a challenge.

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Conflict of interest

There is no conflict of interest

REFERENCES

- Anigboro, A. A., Avwioroko, O. J., Ohwokevw, O. A., Pessu, B. & Tonukari, N. J. (2021). Phytochemical profile, antioxidant, α -amylase inhibition, binding interaction and docking studies of *Justicia carnea* bioactive compounds with α -amylase. *Biophys Chem.* 269(1), 106529. <https://doi.org/10.1016/j.bpc.2020.106529>
- Badami, S., Aneesh, R., Sankar, S., Sathishkumar, M. N., Suresh, B. & Rajan S. (2003). *Antifertility activity of*

- Derris brenipes variety coriacea*. *J. ethnopharmacology*, 84, 99–104.
- Corrêa, G. M., de C. & Alcântara, A. F. (2012) Chemical constituents and biological activities of species of *Justicia* – A Review. *Revista Brasileira de farmacognosia*, 22, 220–238.
- Del-Rio, A., Obdulio, B. G., Casfillo, J., Marin, F. G. & Ortuno, A. (1997). Uses and properties of citrus flavonoids. *J. Agric. Journal of agricultural and food chemistry*, 45(12), 4505–4515.
- Ganapth, S. R., Kumar, D. S., Harami, A., Pathiban, M. P. & Venkateshwarlu, G. (2011). Comparison studies of phytochemical screening. *Asian Journal of Pharmaceutical and Health Sciences*, 1(3).
- GBIF Secretariat (2021). GBIF Backbone Taxonomy-*Justicia carnea* Lindl. Checklist dataset. <https://doi.org/10.15468/39omei>
- Harbron, J. B. (1998). *Phytochemical Methods: A Guide to modern Techniques of plant analysis* (3rd). London: Chapman and Hall Publication.
- Igbinauwa, P. O., Kabari, K. M., & Chikwue, T. C. (2019). Phytochemical and anti-anaemic properties of ethanol leaf extract of *Justicia carnea* Vahl (Acantheaceae). *Nigerian Journal of Pharmaceutical and Applied Science Research*, 8(2), 55–61.
- Kumari, S., Harjai, K., & Chibber, S. (2010). Tropical treatment of *Klebsiella pneumonia* mice using natural products in developing countries. *The Journal of Infection in Developing Countries*, 4(6), 367–377.
- National Population Census (2006). National Population Commission Office Magami road, Jalingo, Taraba State.
- Okonkwo, E. E. (2012). Traditional healing systems among Nsukka Igbo. *Journal of Tourism and Heritage Studies*, 1(1), 69–81.
- Okwu, D. E. & Okwu, M. E. (2004). Chemical composition of spondiasmombin Linn plant parts. *Journal Sustain Agric. Environ.* 6(1), 140–147.
- Okwu, D. E. (2004). Phytochemicals and vitamin content of indigenous spices of Southeastern Nigeria. *Journal Sustain. Agric. Environ.*, 6(1), 30–37.
- Olakunle, T. P., Akinro, E. B., Agolade, J. O., Yoyinoye, A. S., Ghazal, M. A. & Oladeji, S. S. (2013). Inhibitory effect of pure honey on microorganisms isolated from wound. *Journal of Environmental Science, Toxicology and Food Technology*, 3(3), 80–84.
- Olufunke, F. (2021). Nigeria's medicinal plant: *Justicia carnea* (Ewe eje). Nigeria Punch, Healthy living.
- Onyeabo, Ch., Achi, N. K., Ekeleme-Egedigwe, Ch. A., Ebere, Ch. U., & Okoro, Ch. K. (2017). Haematological and biochemical studies on *Justicia carnea* leaves extract in phenylhydrazine induced-anemia in albino rats. *Acta Scientiarum Polonorum Technologia Alimentaria* 16(2), 217–230. <https://doi.org/10.17306/J.AFS.2017.0492>
- Orjiakor, C. A., Uroko, R. I., Njoku, O. U., Ezeanyika, L. U. S., & Uchenna, S. (2019). Nutritive properties of aqueous extract *Justicia carnea* leaves and its effects on haematological and some biochemical indices of anaemia induced male wistar albino rats. *Biomedical Research*, 30(4), 645–654. <https://doi.org/10.35841/biomedicalresearch.30-18-666>
- Osho, A. O., & Bello, O. O. (2010). Antimicrobial effect of honey produced by *Apis mellifera* on some common human pathogens. *Aisan J. Exp. Biol. Sci.*, 1(4), 875–880.
- Radhika, J., Sathya, S., Jothi, G., & Japasheba, J. L. (2013). Cardioprotective role of *Justicia traquebarensis* Linn. leaf extract in isoproterenol induced myocardial infarction in albino rats. *Journal of applied pharmaceutical science*, 3(4), 124–128.
- Salah, N., Miller, N. J., Pagange, G., Tijburg, L., Bolwell, G. P., Rice, E., & Evans, C. (1995). Polyphenolic flavonoids as scavenger of aqueous phase radicals as chain breaking antioxidant. *Arch. Biochem. Biophys.*, 2, 339–346.
- Shyamapada, M., & Manisha Deb M. (2011). Honey: Its medicinal property and antibacterial activity, *Asian Pac. J. Biomed.*, 1(2), 154–160.
- Sini, S., Prashy, P., & George, S. (2018). Preliminary phytochemical investigation and estimation of polyphenolics in different parts of selected species of *Justicia*. *European Journal of Biomedical and Pharmaceutical Sciences*, 5(4), 460–464
- Stray, F. (1998). *The Natural Guide to Medicinal Herbs and Plants*. London; Tiger Books International.
- Topographic Sheet (1968). By Federal Surveyors Nigeria. Topographic Sheet, No. 255, edition 1, with scale of 1, 100,000.
- Trease, G. E. and Evans, W. C. (2009). *Pharmacognsy*. 11th ed. Brailliar Tiridel Can. Macmillian publishers.
- USDA PLANTS (2007–2010). *Justicia carnea*. Updated for ITIS by the Flora of North America Expertise Network.