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Chitta Padmavathi
Department of Biotechnology Acharya Nagarjuna University Nagarjuna Nagar, Guntur-522510, A.P. India.

Kumara Narendra
Department of Biotechnology Acharya Nagarjuna University Nagarjuna Nagar, Guntur-522510, A.P. India.

Doddapaneni Siva Durga Suman Joshi
Department of Biotechnology Acharya Nagarjuna University Nagarjuna Nagar, Guntur-522510, A.P. India.

Kalakota Venkata Naga Rathnakar Reddi
Department of Biotechnology Acharya Nagarjuna University Nagarjuna Nagar, Guntur-522510, A.P. India.

Katta Meera Sowjanya
Department of Biotechnology Acharya Nagarjuna University Nagarjuna Nagar, Guntur-522510, A.P. INDIA.

Jangala Swathi
Department of Biotechnology Acharya Nagarjuna University Nagarjuna Nagar, Guntur-522510, A.P. India.

Alapati. Krishna Satya
Assistant Professor and Coordinator Department of Biotechnology Acharya Nagarjuna University Nagarjuna Nagar, Guntur-522510, A.P. India.

Correspondence:
Alapati. Krishna Satya
Assistant Professor and Coordinator Department of Biotechnology Acharya Nagarjuna University Nagarjuna Nagar, Guntur-522510, A.P. India.

Active ingredient estimation and *in-vitro* analysis of *Jacquinia barbasco* (Loefl.) Mez plant extracts

Padmavathi CH, Narendra K, Suman Joshi DSD, Rathnakar Reddi KVN, Sowjanya KM, Swathi J, Krishna Satya A

Abstract
Jacquinia barbasco (Loefl.) Mez is a native genus with 35 species in Central America and the Caribbean. These plants are used to cure stomach pain in some rural areas. The qualitative and quantitative phytochemical analysis helped us in finding out the active ingredients, and many of the *in vitro* activities of this species are carried out to assay its bioactive nature. The crude extracts were screened for the presence or absence of secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins, and anthraquinone using standard procedures. The total phenol contents were determined by the Folin Ciocalteu procedure. Agar well-diffusion method was followed to determine the antimicrobial activity. The free radical scavenging activity of the extract was measured by using 1, 1-Diphenyl-2-Picrylhydrazyl radical (DPPH). The results of preliminary phyto chemical screening confirmed the presence of various classes of secondary metabolites in the *Jacquinia barbasco* (Loefl.) Mez plant extract. The extracts showed better free radical scavenging activity and antimicrobial activity.

Keywords: *Jacquinia barbasco* (Loefl.) Mez, Qualitative Analysis, Total Phenol content, Antimicrobial Activity, Antioxidant Activity.

1. Introduction
Jacquinia barbasco (Loefl.) Mez is a shrub belonging to the family *Theophrastaceae*. It has an evergreen life cycle with leaves throughout the year. It is usually 4.5 m in height and 15 cm in stem diameter. It has a bushy nature. *Jacquinia barbasco* (Loefl.) Mez is also known as torch wood, azucarer, and bizcocho. The outer bark is dark brown in colour and thinly fissured and the inner bark is light brown and bitter. The wood is hard and light brown in colour. The crowns are compact and often wind-hedged in their sea-sidem habitat. Leaves are a bit stiff, thick and pale yellow or green in colour. They are stipulate or obovate, alternate or in threes, and clustered at the ends of the twigs.

It has a regularly thickened rootstalk and few stems. The bark is dark brown and thinly fissured, and the inner bark is light brown and bitter. The wood is light brown or yellowish and hard. The crowns are compact and often wind-hedged in their sea-sidem habitat. The thick and stiff, dull yellow-green leaves are spatulate or obovate, alternate or in threes, and clustered at the ends of the twigs. Terminal racemes are several to many-flowered. The small, white or yellowish, five-lobbed flowers are fragrant. The fruit (berries) are globose 8 to 12 mm in diameter, orange-red, and contain one to four rounded, brown seeds [1, 2, 3].

Jacquinia barbasco (Loefl.) Mez is a genus of 35 species in Central America and the Caribbean. These plants are used to cure stomach pain in some rural areas. The active ingredients in the plant part used have varying potencies and modes of action depending on the mode of application, it may be directly or in the form of extract, polar or non-polar [4].The qualitative and quantitative phytochemical analysis helps us in finding out the active ingredients, and many of the *in vitro* activities of this species are carried out to assay its bioactive nature.

2. Material and Methods
2.1 Plant collection
The aerial parts of the plant were collected from the herbal garden of the Acharya Nagarjuna University, Guntur. The plant was identified accordingly to various literatures, including other pertinent taxonomic literature.

2.2 Collection of microorganisms
The microorganisms were collected from the Department of Biotechnology, Acharya Nagarjuna University, Guntur and they were reconfirmed by gram staining and sub cultured in appropriate selective media.

2.3 Extraction procedure
The aerial parts of *Jacquinia barbasco* (Loefl.) Mez were carefully separated, cleaned, shade dried, mechanically grinded and coarsely powdered. The powder was subjected to solvent extraction with Methanol, Hexane, Acetone, and Water. The Extracts were concentrated by using the Rotary Evaporator and the yield of the Extract was noted with respect to the dried plant material.

2.4 Qualitative Phytochemical Analysis [5, 6, 7, 8]
Standard screening tests of four extracts were carried out for various plant constituents. The crude extracts were screened for the presence or absence of secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins, and anthraquinone using standard procedures.

2.5 Antimicrobial activity
Agar well-diffusion method was followed to determine the antimicrobial activity. Nutrient agar (NA) and Sabouraud Dextrose Agar (SDA) plates were swabbed (sterile cotton swabs) with 24 hours old - broth culture of respective bacteria and fungi. Four wells (10mm diameter) were made in each of these plates using sterile cork borer. About 50µl of plant extracts were added using sterilized dropping pipettes into the wells and allowed to diffuse at room temperature for 2 hours. The plates were incubated at 37 °C for 18-24 hours for bacterial pathogens and 28 °C for fungal pathogens. Respective solvent control for leaf extracts was also maintained and the diameter of the zone of inhibition was recorded in mm and compared with standard values.

2.6 Determination of total phenols
The total phenol contents were determined by the Folin Ciocalteu procedure by Skerget *et al*. 2005 [9]. Briefly, different concentrations of the extracts were taken to that 0.1 ml of Folin Ciocalteu reagent and 2.5 ml of 0.2N Na_2CO_3 were added and incubated for 30 min at room temperature. Distilled water was used as blank. Absorbance was measured at 760 nm using Thermo Fisher double beam spectrophotometer. Gallic acid was used as standard and the results were expressed as µg of gallic acid equivalents per gram dry mass of extract (µg GAE gDM).

2.7 Antioxidant Properties: Brand-Williams, W1995 [10]
1, 1- Diphenyl-2-Picrylhydrazyl radical (DPPH) scavenging activity:
The free radical scavenging activity of the extract was measured by using 1, 1- Diphenyl-2-Picrylhydrazyl radical (DPPH) as described by Brand- Williams, W (1995) [10] with some modifications. The extracts were prepared at 1mg/ml concentration with DMSO solution. The mixture was made uniform and 100-500µg/ml concentrations were made as working solutions. Further 0.004% (W/V) solution of DPPH in methanol was added to the solution. The mixture was shaken and incubated for 60min in the dark at room temperature. The absorbance was measured at 517 nm against blank. The DPPH scavenging activity (I %) was calculated as follows:

$$I\% = \left[\frac{(Ao - As)}{Ao}\right] \times 100$$

Where **Ao** is the absorbance of the DPPH solution without sample extract and as is the absorbance of sample with DPPH solution.

3. Results
3.1 Yield of Extract
In the present study the yield of crude extract was obtained by measuring its dry weight. Yield was found to be low in hexane extract due to its low polarity and the yield was found to be more in methanol (29.268%). The color of the extract was found to be dark brown in hexane extract, dark green in acetone and methanol extracts and dark red in aqueous extract it was shown in table 1 & figure 1.

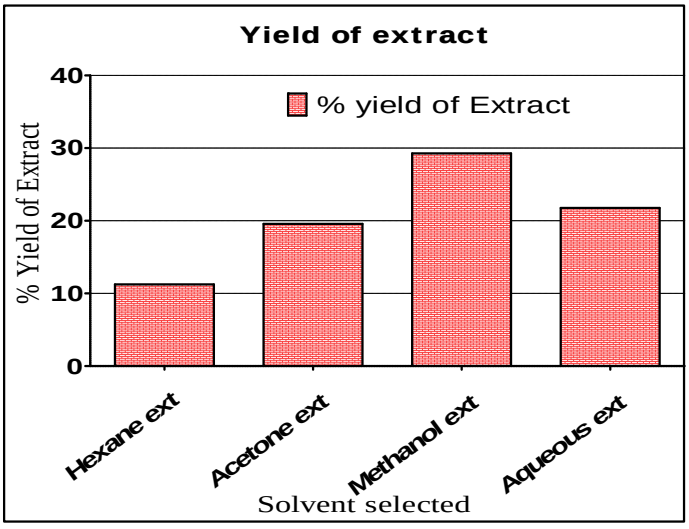

Fig 1: Graphical representation of yield obtained from the different solvent systems

Table 1: Physico - Chemical Evaluations of *Jacquinia barbasco* (Loefl.) Mez

Solvent	Initial Weight of the Powder (gm)	Final Weight of the Powder (gm)	Weight of the Crude Extract (gm)	Crude Extract %	Colour of the Extract
Hexane	50	44.384	5.616	11.232	Dark Brown
Acetone	50	40.215	9.785	19.57	Dark Green
Methanol	50	35.366	14.634	29.268	Dark Green
Aqueous	50	39.121	10.879	21.758	Dark Red

3.2 Phytochemical Analysis

The results of preliminary phytochemical screening were given in table 2. It shows the presence of alkaloids, phenols, flavonoids, steroids, tannins, saponins and reducing sugars. Reducing sugars, alkaloids, phenols, tannins and flavonoids were present in methanol, acetone and aqueous extracts except hexane extract. Coumarins, anthocyanins, leucoanthocyanins were completely absent in all the extracts.

Table 2: Phytochemical Analysis of Arial Part Extracts of *Jacquinia barbasco* (Loefl.) Mez

***Jacquinia barbasco* (Loefl.) Mez**					
S. No	**Tests**	**Hexane Extract**	**Acetone Extract**	**Methanol extract**	**Aqueous extract**
01.	Alkaloids				
	Mayers	Negative	Positive	Positive	Positive
	Dragon	Negative	Positive	Positive	Positive
	Wagners	Negative	Positive	Positive	Positive
	Hagers	Negative	Positive	Positive	Positive
02.	Phenolics				
	$Fecl_2$ Test	Negative	Positive	Positive	Positive
03.	Flavonoids				
	Lead Acetate Test	Negative	Positive	Negative	Positive
	NaOH Test	Negative	Positive	Positive	Positive
	Ethyle acetate Test	Negative	Negative	Negative	Positive
04.	Anthraquinone Test				
	Borntrager's Test	Negative	Negative	Positive	Positive
05.	Steroids				
	Salkowski's Test	Negative	Positive	Positive	Positive
06.	Tannins				
	$Fecl_2$ Test	Negative	Positive	Positive	Positive
	Lead acetate Test	Negative	Positive	Positive	Positive
	Pot. dichromate Test	Negative	Positive	Positive	Positive
07.	Saponins				
	Vigorous Shaking Test	Negative	Positive	Negative	Positive
08.	Anthocyanins				
	Ammonia-HCl Test	Negative	Negative	Positive	Negative
09.	Leuco-Anthocyanin				
	Iso Amyl Alcohol Test	Negative	Negative	Negative	Positive
10.	Coumarins				
	NaOH Test	Negative	Negative	Positive	Positive
11.	Reducing Sugars				
	Keller-Kiliani Test	Negative	Positive	Positive	Positive

3.3 Antimicrobial screening

The antimicrobial activity of hexane, acetone, methanol, and aqueous extracts of *Jacquinia barbasco* (Loefl.) Mez against ten bacterial and four fungal strains was tested. They have shown moderate to high activity against the tested organisms. Among them methanol extract showed high antimicrobial activity followed by acetone and aqueous extracts. Hexane showed low antimicrobial activity when compared with the other three extracts. It was shown in figure 2 & 3.

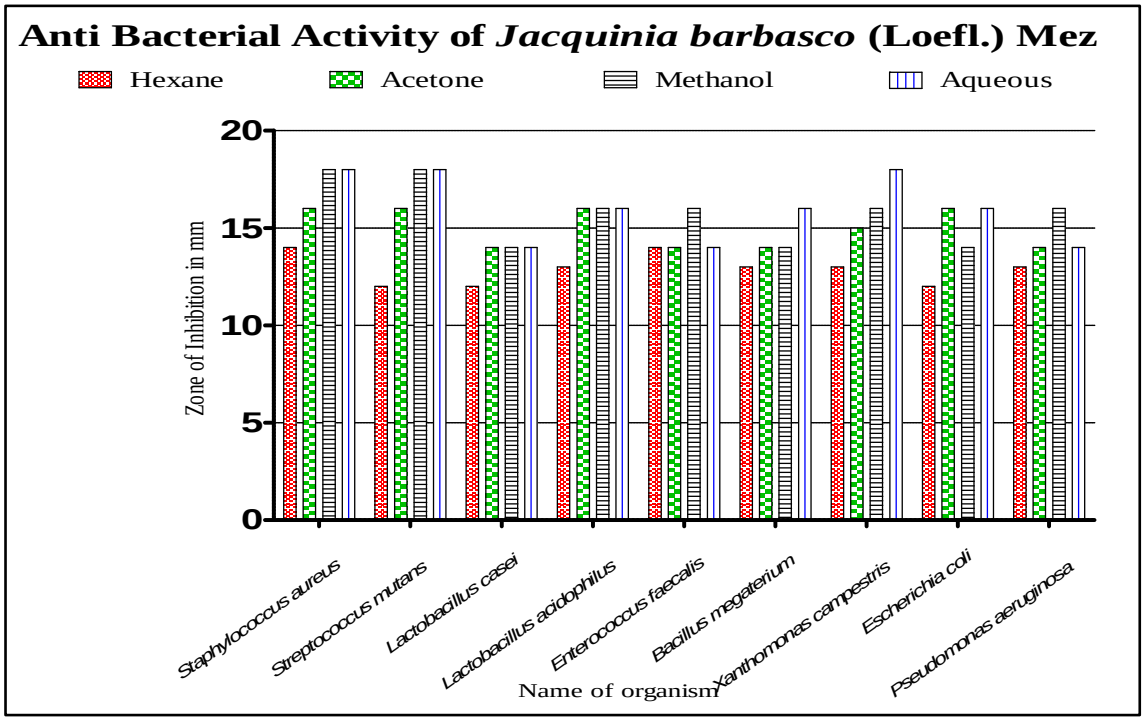

Fig 2: Antibacterial Activity of *Jacquinia barbasco* (Loefl) Mez

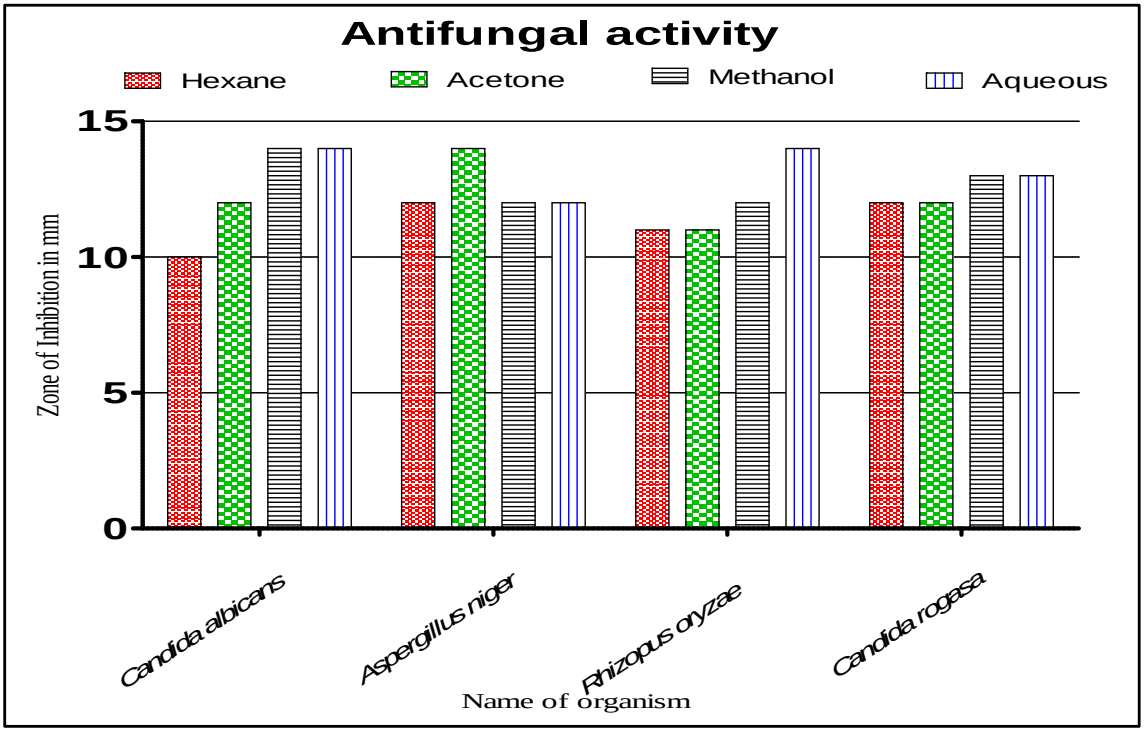

Fig 3: Antifungal Activity of *Jacquinia barbasco* (Loefl.) Mez

3.4 Phenol Quantitative Determination and antioxidant activity

The results of total phenolic content and antioxidant activity of various extracts of *Jacquinia barbasco* (Loefl.) Mez has revealed that the methanol extract of this plant showed highest amount of phenol compounds as well as the best antioxidant activity in DPPH assay followed by water, acetone and hexane extract. It was shown in Figure 4&5.

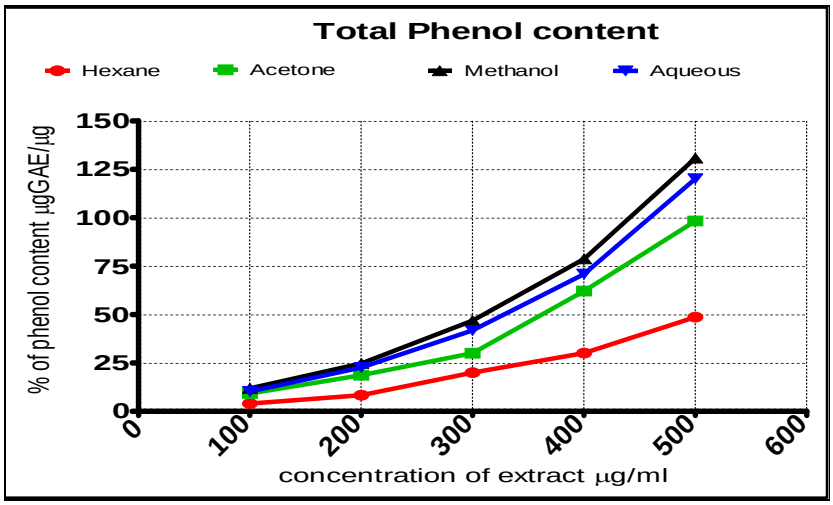

Fig 4: Total Phenol content of *Jacquinia barbasco* (Loefl.) Mez

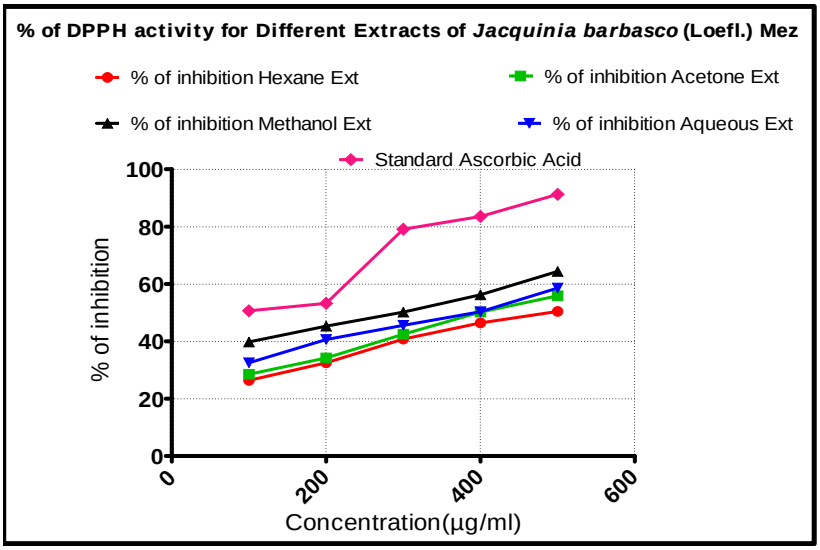

Fig 5: DPPH Antioxidant Activity of *Jacquinia barbasco* (Loefl.) Mez

4. Discussion

Medicinal plants since ancient time are praised for their diverse pharmacological actions which could be attributed to the presence of secondary plant metabolites such as alkaloids, flavonoids, glycosides, tannins, steroids *etc.* Some of these plants are important sources of natural antioxidants that have been shown to reduce the risk and progression of certain acute and chronic diseases such as cancer, heart diseases and stroke by scavenging free radicals which are implicated in the pathogenesis of many diseases

The results of preliminary phytochemical screening confirmed the presence of various classes of secondary metabolites in the *Jacquinia barbasco* (Loefl.) Mez plant extract.

DPPH radical scavenging activity of *Jacquinia barbasco* (Loefl.) Mez plant extract was compared with standard ascorbic acid in this study. Although standard antioxidant had higher scavenging activity at all tested concentrations than the extract, the extract still showed good free radical scavenging activity. The free radical scavenging property of *Jacquinia barbasco* (Loefl.) Mez may be one of the mechanisms by which this plant is effective as a traditional medicine. The consumption of the *Jacquinia barbasco* (Loefl.) Mez plant extract can be beneficial in preventing oxidative stress related degenerative diseases.

The results of our study suggest that *Jacquinia barbasco* (Loefl.) Mez plant extract are rich in bioactive compounds and have good biological activities. However, further detailed investigations are needed to ascertain the mechanisms and constituents behind its biological actions.

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