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Phytochemical screening and antimicrobial potential of ethanolic fruit extract of *Cryptolepis Buchanani* (Roem and Schult)

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Abstract
Cryptolepis buchanani (Asleclepiadaceae) is a climbing shrub commonly known as jambupatra sariva and Karanta, The leaves is widely used in folk medicine in Southeast Asia. The ethanolic extract of fruits of this plant. The aim of the present study was to investigate the antibacterial and antifungal potential of the ethanolic extract of *Cryptolepis buchanani* against some pathogenic bacteria and fungi. It is observed that the ethanolic extract of *cryptolepis buchanani* effective biological activity. Phytochemical screening confirmed the presence of flavonoids, tannins, phenols, saponins, steroids, proteins and carbohydrates in ethanolic fruit extract.

Keywords: phytochemical, antimicrobial, fungi, extracts, medicine

Introduction
From ancient times plants are recognized as a major resource for mankind. Plants are used as food material, in cosmetics and the mostly in medicine. From human civilization up to date plants are primary resources of medicines. Different people used plants in various ways for medicinal purposes. Millions of the people in the third world use herbal medicines because they believe in them and regard them as their own system of medicine [1]. The use of herbal medicine, the dominant form of medical treatment in developing countries, has been increasing in developed countries in recent years [2]. According to World Health Organization (WHO), about 25% of modern medicines are descended from plants first used traditionally. Many others are synthetic analogues built on prototype compounds isolated from plants [3]. *Cryptolepis buchanani* Roem. & Schutt, locally called Dudhi vel in Maharashtra, which belongs to the family Asclepiadaceae. It is a large climbing or straggling shrub. Leaves 2-5 cm long, elliptic oblong, opposite green shining above with a pale whitish beneath. Flowers yellowish green in axillary cymes and flowering occurs in June. In ayurvedic practice, the root is used as a substitute for that of *Hemidesmus indicus* to treat gout, polyuria, wounds and leprosy. It is considered alterative refrigerant and tonic [4]. In last three decades numbers of new antibiotics have produced, but clinical efficacy of these existing antibiotics is being threatened by the emergence of multi drug-resistant pathogens [5]. In general, bacteria have the genetic ability to transmit and acquire resistance to drug [6]. Antibiotics are the main basis employed in the treatment of different microbial diseases. On the basis of evidence of the rapid global spread of drug-resistant microbes, the need to find new antimicrobial agents has great importance [7]. The present investigation was carried to evaluate antibacterial and antifungal efficacy of the ethanol extract of fruits of *C. buchnani* Roem. & Schult.

Materials and Methods
Collection of plant material
The fresh fruits of *Criyptolipis buchnani* Roem & Schult was collected from the various localities of Nanded, Maharashtra, India. Fruits were splashed with distilled water and dried in a shaded area at room temperature for a period of a week. Then the dried fruits were grinded with an mixer blender and then sieved.

Preparation of plant extracts
The extracts of fruits in ethanol were prepared. The 50 gm of dried powder was extracted with 300 ml solvent using Soxhlet apparatus for 24 hrs. The extract were lyophilized and stored in 4^{O} C.

Preliminary Qualitative screening of plant extracts
Standard protocols were used for the phytochemical analysis. Phytochemical screening for the presence of major types of compounds in the extract was done by Harborne [8] with some modifications.

- **Test for protein**

Ninhydrin test
Crude extract when boiled with 1ml of 0.2% solution of ninhydrinn, violet colour is appeared suggesting the presence of amino acids and protein.

- **Test for carbohydrates**

Fehling's test
Equal of Fehling A and Fehling B reagents were mixed together and 1ml of sample was added to extracts and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicate the presence of reducing sugars.

Benedict's test
Extract when mixed with 1 ml of Benedict's reagent and boiled, reddish brown precipitate formed which indicate the presence of carbohydrates.

Iodine test
Extract were mixed 1ml of iodine solution respectively .A dark blue or purple coloration indicates the presence of carbohydrate.

- **Test for phenols and tannins**

The extract was mixed with 1 ml of 1% solution of FeCl3 respectively. A blue-green or black coloration indicates the presence of phenols and tannins

- **Test for flavonoids**

Shinoda test
Each extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Pink scarlet color appeared after few minutes which indicated the presence of flavonoids.

Alkaline reagent test
Each extracts were with 1ml of 1%of solution of NaOH respectively. An intense yellow color was formed which turned colorless on addition of few drops of dilute acid which indicated the presence of flavonoids.

- **Test for saponins**

Extract were mixed with 5ml of distilled water in respective test tubes and were shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins

- **Test for glycosides**

Liebermann'test
Extract were mixed with each of 1ml of chloroform and 1ml of acetic acid. The mixture was cooled in ice. Carefully concentrated H2SO4 was added.
A colour changes from violet to blue to green indicated the presence of steroidal nucleus, i.e, and glycone portion of glycoside.

Salkowaski's test
Extract were mixed with 1ml of chloroform. Then 1ml of concentrated H2SO4 was added carefully and shaken gently. A reddish brown colour indicated presence of steroidal ring i.e., glycone portion of the glycoside.

- **Test for steroids**

Extract were mixed each of 1ml of chloroform and concentrated H2SO4 was added sidewise. A red color produced in the lower chloroform layer indicates the presence of steroids.
Another test was performed by mixing extracts with 1 ml of chloroform. Then 1ml of each concentrated H2SO4 was added and acetic acid was poured into the mixture. The development of a greenish coloration indicates the presence of steroids.

- **Test of terpenoids**

Extract were dissolved in 1ml of chloroform and evaporated to dryness. To this 1ml concentrated H2SO4 was added and heated for couple of minute's .A grayish colour indicates the presence of terpenoids.

- **Test for alkaloids**

Extract were mixed with 1ml of 1% HCl and gently heat. Mayer's and Wagner's reagents were then added to the mixture. Turbidity of resulting precipitate was taken as evidence for the presence of alkaloids.

- **Test for phlobatannins**

1ml of aqueous extract was added to 1ml of HCL and the mixture was boiled. Deposition of red precipitate was taken as an evidence for the presence of phlobatannins.

- **Test for fixed oil and fatty acid**

Spot test
Prepare a spot on the filter paper with the help of test solution and oil staining on the filter paper indicates presence of fixed oil and fatty acid.

Micro-organisms
In the present investigation, the antibacterial and antifungal activity of ethanolic extract were tested against bacteria and fungi. For antibacterial activity, four bacteria are selected viz. *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Bacillus sp.* Five species of fungi viz. *Haleminthosporum graminium*, *Aureobasidium pallulans*, *Alternaria solani*, *Aspergillus fumigatous* and *Aspergillus nodulum* used for antifungal activity.

Antifungal Screening
The antifungal activity of the fruit extract was evaluated using Agar Well Diffusion Method of Jahan. [9] With minor modifications**.** Plates were prepared with 20ml of potato dextrose agar. Fungal suspension was spreaded on the surface of PDA plate by using sterile glass rod. All the culture plates were allowed to dry for 5min. wells were cut out using cork borer and filled 100 µl of fruit extract. Standard antibiotic like flucanozole is used as positive control and methanol as negative control. Then these plates were kept in refrigerator for diffusion for 10-15 min. The plates were incubated at 37 °C for 24-48 hrs and zone of inhibition was observed.

Antibacterial Screening
The antibacterial activity of *Cryptolepis buchanani* Rorm. and Schult was performed by agar well diffusion method of Jahan [9] against selected bacteria. Plates were prepared with 20 ml of nutrient agar media and bacterial culture was spread on the surface of NA plate by using sterile glass rod. All the culture plates were allowed to dry for about 5min. Wells were cut out using cork borer and filled with 100 µl of fruit extract, standard antibiotic like streptomycin is used as positive control and ethanol is used as negative control. Then these plates were kept in refrigerator for diffusion for 10-15 min. the plates were incubated at 37 °C for 24hrs zone of inhibition was observed.

Results and Discussion
Preliminary phytochemical analysis of fruits of *Criyptolipis buchnani* Roem & Schult was carried out by using ethanolic extract. The fruits extract showed the presence of flavonoids, tannins, phenols, saponins, steroids, proteins and carbohydrates. (Table1)

Table 1: Phytochemical Analysis of fruit extract of *Criyptolipis buchnani* Roem & Schult.

Phytochemical	Test	Extract
Alkaloids		--
Carbohydrate	Fehling's test	+
	Benedicts test	+
	Iodine test	+
Fixed oil and Fatty acid		--
Flavonoids	Shinoda	+
	Alkaline reagent	--
Glycoside	Liebermann's test	--
	Salkowaski's test	--
	Killer-Kilani test	--
Phenols and Tannins	FeCl3 test	+
Protein	Ninhydrine test	+
Saponins		+
Phlobatannins		-
Terpenoids		-
Steroids		+

*Present (+), Absent (--)

Table 2: Antibacterial activity of *Criyptolipis buchnani* Roem & Schult fruit extracts

Extract	Zone of inhibition in mm			
	Escherichia coli	*Staphylococcus aureus*	*Bacillus subtilis*	*Bacillus sp.*
Ethanol	09	08	10	11

The antibacterial activity of *Criyptolipis buchnani* Roem & Schult fruit extract were examined against different bacteria. The ethanolic extract showed the higher zone inhibition against *Bacillus sp.* While least inhibition showed against the *E. coli.* The overall fruit extract showed high antimicrobial response against bacteria.

Table 3: Antifungal activity of *Criyptolipis buchnani* Roem & Schult fruit extracts

Extract	Zone of inhibition in mm				
	Haleminthosporum graminium	*Aureobasidium pallulans*	*Alternaria solani*	*Aspergillus fumigatous*	*Aspergillus nodulum*
Ethanol	14	R	R	7	10

The antifungal activity of *Criyptolipis buchnani* Roem & Schult fruit extract were examined against different fungi. Ethanolic extract showed the higher zone inhibition against *Haleminthosporum graminium* while *Aureobasidium pallulans* and *Alternaria solani* showed resistance against fruit extract. The overall extract showed good antifungal response against selected fungi.

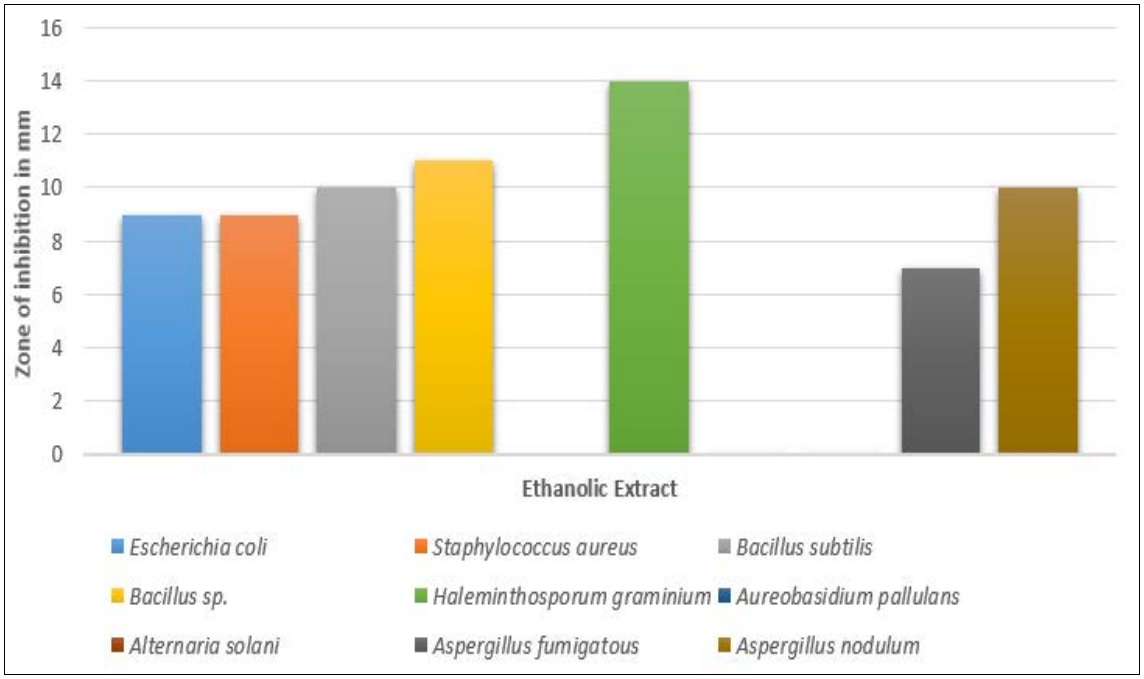

Fig 1: Antimicrobial properties of *Criyptolipis buchnani* Roem & Schult fruit extracts

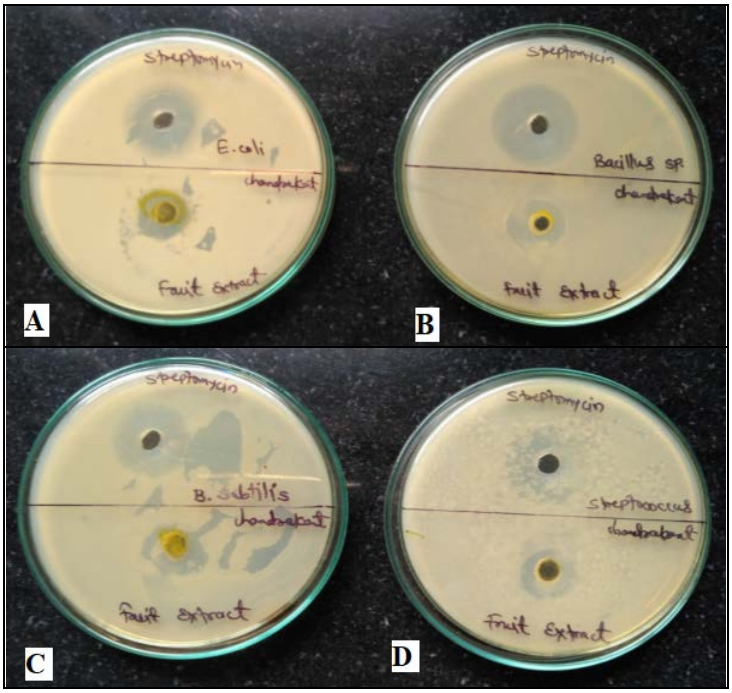

Fig 2: The antibacterial activity of *Criyptolipis buchnani* Roem & Schult ethanolic fruit extract against *Escherichia coli*: A, *Bacillus sp.*: B, *Bacillus subtilis*: C and *Staphylococcus aureus*: D

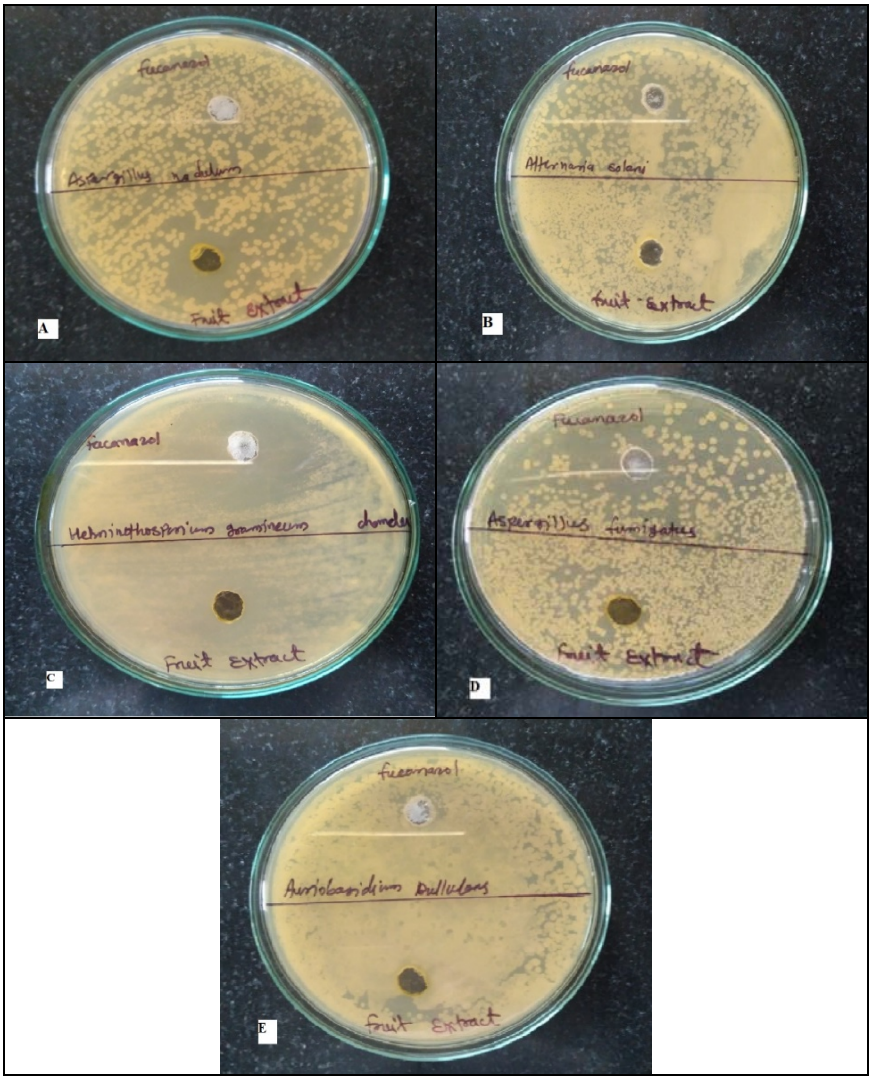

Fig 3: The antifungal activity of *Criyptolipis buchnani* Roem & Schult ethanolic fruit extract against *Aspergillus nodulum*: A, *Alternaria solani*: B, *Haleminthosporum graminium*: C, *Aspergillus fumigatous*: D, and *Aureobasidium pallulans*: E.

Conclusion

Preliminary qualitative phytochemical analysis of the *Criyptolipis buchnani* Roem & Schult fruit extract revealed the presence of flavonoids, tannins, phenols, saponins, steroids, proteins and carbohydrates. These phytoconstituents or secondary metabolites are reported to have many biological and therapeutic properties, so this species is expected to have many medicinal uses. The *Criyptolipis buchnani* Roem & Schult ethanolic fruit extract shows potent antibacterial and antifungal activity against selected bacteria and fungi. So this plant species can be expected to have many therapeutic uses and can be further studied for the production of antibiotics and pharmaceutical drugs.

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