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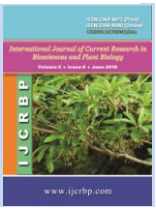

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Antimicrobial Activity of Ethnomedicinally Important Asclepiads from Shervaroyan Hills, Southern Eastern Ghats

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ABSTRACT

Based on the ethnomedicinal data and availability, six plant species, *Cryptolepis buchanani, Gymnema sylvestre, Hemidesmus indicus, Secamone emetica, Leptadenia reticulata* and *Wattakaka volubilis* belonging to the family Asclepiadaceae from Shervaroyan Hills, Southern Eastern Ghats have been selected for the present study. The leaf materials of the selected ethnomedicinal plants were used for cold extraction with ethanol and the crude ethanolic leaf extracts at 1000ppm concentration were used for antimicrobial assay. Using antimicrobial disc diffusion method, antibacterial activity of the leaf extracts were tested against *Bacillus subtilis* and *Escherichia coli*; antifungal activity was tested against *Candida albicans.* The results of the present study revealed that the ethanolic leaf extracts of *Cryptolepis buchanani* showed highest zone of inhibition of 2.3 cm diameter against *Bacillus subtilis.* The leaf extracts of *Hemidesmus indicus* exhibited a maximum inhibitory activity against *Escherichia coli* with a zone of inhibition of 2.8 cm. The highest zone of inhibition diameter in *Leptadenia reticulata* leaf extract was 1.4 cm recorded against the fungus, *Candida albicans*. There is a scope to use ethanolic extracts of the leaves of the Asclepiads, used in the study, against bacteria and fungus.

Introduction

Traditionally, herbal folklorists and in Indian systems of medicines namely *Ayurveda*, *Unani* and *Siddha* has played a vital role in elementary diseases. Ethnobotany of medicinal plants have been playing important role in health care systems of ethnic communities. Shervaroyan Hills are in the range of Eastern Ghats situated in Salem district in Tamil Nadu. It is situated at an altitude range of 300 to 1600 meters above sea level and the highest point is the Servarayan temple at 1.623 m. They are located between 11^0C 45’ 56” N latitude and 78^0C 17’ 55” E longitude. The forest types range from every green to moist deciduous (Champion and Seth, 1968). Ethnobotanical studies in the Eastern

Ghats of Tamil Nadu have been carried out earlier by several researchers (Muralidhara Rao and Pullaiah, 2007; Kottaimuthu, 2008; Kadavul and Dixit, 2009; Franxis Xavier et al., 2011). A perusal of the available literature reveals that information on the comprehensive survey, documentation and enumeration of wild medicinal plants by the indigenous people in the Shervaroyan hills of Eastern Ghats is meager and there is no such comprehensive study on this region especially with reference to Asclepiads-an important group of medicinal plants largely used by tribal and non-tribal communities.

A literature survey on ethnomedicine and folk medicine says that about 3,000 materials from 2,764 plant species have been screened for their pharmacological and ethnotherapeutic properties (Anonymous, 1998). But still a vast wealth of medicinal plants has not been explored which contains active medicinal properties of curing a number of killer diseases like cancer, HIV, etc. Asclepiadacean medicinal plants from Dindigul district, Tamil Nadu has been screened for antimicrobial activities by Karuppusamy et al. (2001). However, ethnomedicinally important Asclepiads in Shervaroyan hills, southern Eastern Ghats is *hitherto* unexplored. Hence the present study has been carried out with the following objectives: (1) To survey the ethnomedicinal plants with special reference to the plants belonging to the family, Asclepiadaceae in Shervaroyan hills, southern Eastern Ghats, (2) To collect and to prepare the ethanolic extracts of the leaves of selective ethnomedicinal Asclepiads from Shervaroyan hills, southern Eastern Ghats and (3) To study the antimicrobial activity of ethanolic extracts of ethnomedicinal plants against a fungus, *Candida albicans* and two species of bacteria, *Bacillus subtilis* and *Escherichia coli*.

Materials and methods

Selection and collection of ethnomedicinal plants

Based on the preliminary survey made on the ethnomedicinal plants of the family, Asclepiadaceae in Shervaroyan hills (southern Eastern Ghats), Tamil Nadu and on the availability of the plants, the following plants were selected. The list of plants selected for the study is given below (Figs. 1, 2 and 3). The leaves of the medicinal plants selected for the present study were collected from Shervaroyan hills (Eastern Ghats), Tamil Nadu and the identification was confirmed using standard local floras (Gamble and Fischer, 1957; Matthews, 1983). The leaves collected were immediately transported to the laboratory for further processing.

Cryptolepis buchanani Roemer & Schultes (Fig. 1: A-D)
Vernacular (Tamil) names: *Maattaankodi, Paal kodi, Meesaikaaran chedi.*

Gymnema sylvestre (Retz.) R.Br. ex Roemer & Schultes (Fig. 1: E-H)
Vernacular (Tamil) names: *Sirukurinja, Sakkaraikkolli.*

Hemidesmus indicus (L.) R.Br. (Fig. 2: A-C)
Vernacular (Tamil) name: *Nannari.*

Secamone emetica (Roxb.) R.Br. ex Schultes (Fig. 2: D-F)
Vernacular (Tamil) names: *Sagadam, Aangaravalli, Siru aattaankodi, Nilamarandaikodi.*

Leptadenia reticulata (Retz.) Wight & Arn.
Vernacular (Tamil) name: *Paalaikkodi.*

Wattakaka volubilis (L.f) T. Cooke (Fig. 3: A-C)
Vernacular (Tamil) names: *Kodi-palai, Kurinja, Kurinjaakkeerai.*

Preparation of crude extract

The leaves of the plants collected were individually washed with tap water, blotted with filter paper and spread over news paper for air drying under shade. After complete dryness, the leaves of individual plants were powdered using a mixer grinder. A known quantity of leaf powder (100 g) of each plant was taken in a 250 ml conical flask and added

with 100-200 ml of ethanol (95%) (Fig. 3 E). Ethanol was used for the extraction of phytochemicals because it has the ability to dissolve the phytochemical compounds like tannins, polyphenols, flavonols, terpenoids and alkaloids (Silva et al., 1997; Habtenmariam et al., 1993).

The ethanol-leaf powder mixtures were kept at room temperature for 48 hours and stirred using orbital shaker (Fig. 3 D). After 48 hours, the extract of each plant was filtered through Whatmann No.1 filter paper to exclude the leaf powder. Then each filtrate was kept in beaker on a water bath at 45ºC until the solvent gets evaporated. A greasy final material (crude extract) obtained for each plant was transferred to screw cap tubes and stored under refrigerated condition till use.

By using digital electronic balance, 200 mg of each crude extract was carefully taken in a standard measuring flask and 5 ml of ethanol was added to dissolve the extract and one or two drops of emulsifier (Triton-X100) was added to completely dissolve the extract. Then it was made up to 200 ml by adding distilled water. This forms the stock solution of 1000 ppm (i.e., 1mg/ml). For the antifungal and antibacterial assays using individual plant extracts, the stock solution of 1000 ppm concentration was used.

Antimicrobial assay

Two species of bacteria, *Bacillus subtilis* and *Escherichia coli* and a fungal strain, *Candida albicans* were obtained from the Microbial Type Culture Collection (MTTC) of Institute of Microbial Technology (IMTECH), Chandigarh, and were used for the present study. The fungal and bacterial species was maintained by sub-culture method in nutrient agar slants. Kirby-Bauer disc diffusion technique was used to test the sensitivity of selected test organism to the ethanolic leaf extracts (Bauer et al., 1966) (Fig. 3: F-G).

The Petri plates of 100mm diameter with Muller-Hinton agar were swabbed with broth cultures test microbes by using sterile swab. Over this, prepared antimicrobial discs were placed under aseptic conditions. Control sets with standard antibiotic amphotericin (30 µg/disc) for *Candida albicans* and ciprofloxacin (30 µg/disc) for to show the resistance and sensitivity pattern of test organism. Also the discs without plant extract (discs prepared using 200 ml distilled water + 5 ml ethanol + one or two drops of emulsifier) were also maintained as another set of control for the test organism. The plates were then incubated at 37ºC for 24 hours and the zone of inhibition (ZI) was measured in cm and recorded. From the results activity index was calculated by comparing the zone of inhibition (ZI) of leaf extracts with standard antibiotics.

Results and discussion

The antibiotic reference standards for the bacterial strains, ciprofloxacin showed a zone of inhibition of 3.0 cm for both *Bacillus subtilis* and *Escherichia coli* (Table 1). The antifungal standard used for *Candida albicans*, amphotericin showed 2.5 cm zone of inhibition (Table 1).

The results obtained for the antibacterial and antifungal activities of ethanolic leaf extracts of ethnomedicinally important Asclepiads from Shervaroyan hills, southern Eastern Ghats are provided in Tables 2 and 3. The ethanolic leaf extracts of *Cryptolepis buchanani* showed highest zone of inhibition of 2.3 cm against *Bacillus subtilis* followed by *Gymnema sylvestre* (1.9 cm)> *Leptadenia reticulata* (1.7 cm)>*Secamone emetica* (1.6 cm) >*Hemidesmus indicus* (1.5 cm) (Table 2, Fig. 3G). The findings of Sittiwet and Puangpronpitag (2009) is parallel to the present study who reported that the aqueous extracts of *Cryptolepis buchanani* showed inhibitory effect against *Stapylococcus aureus, Escherichia coli, Salmonella typhimurium, Klebsiella pneumoniae, Proteus vulgaris, Bacillus subtilis, Lactobacillus plantarum* and *Streptococcus epidermidis*. The MICs (Minimal Inhibitory Concentrations) and MBCs (Minimal Bactericidal Concentrations) of this plant against all tested bacteria are in the range of 1-16 and 2-32 g L^{-1}, respectively (Sittiwet and Puangpronpitag, 2009).

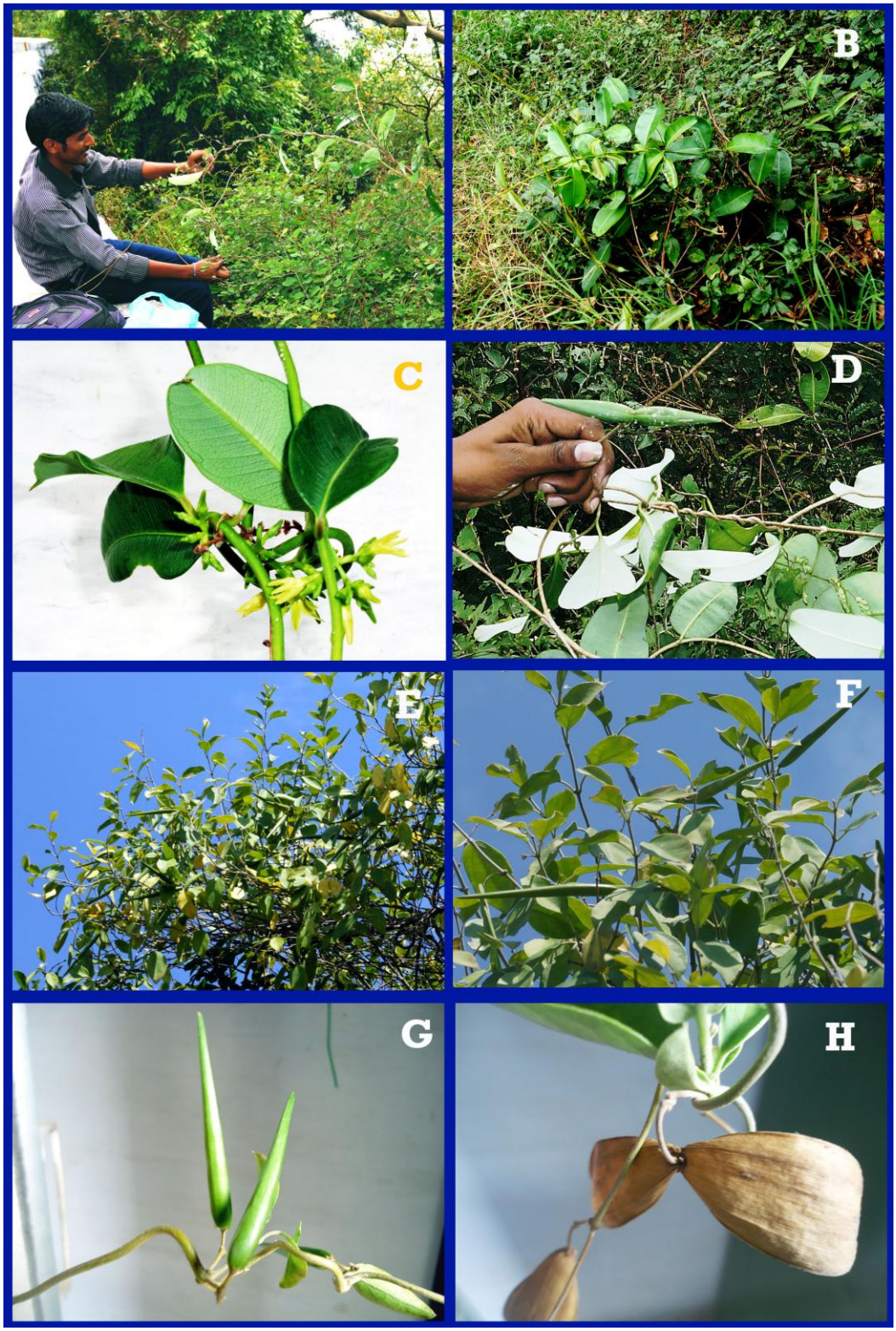

Fig. 1: Collection of ethnomedicinal plants in Shervaroyan hills, southern Eastern Ghats, Salem Dist. A – Collection of plants in Shervaroyan hills; B–*Cryptolepis buchanani* Roemer & Schultes-Habit; C – *Cryptolepis buchanani* Roemer & Schultes – A flowering twig; D – *Cryptolepis buchanani* Roemer & Schultes with follicles; E – *Gymnema sylvestre* (Retz.) R.Br. ex Roemer & Schultes – Habit; F – *Gymnema sylvestre* (Retz.) R.Br. ex Roemer & Schultes – Fruiting stage; G – *Gymnema sylvestre* (Retz.) R.Br. ex Roemer & Schultes – Close view of fruits; H – *Gymnema sylvestre* (Retz.) R.Br. ex Roemer & Schultes – Dehisced follicle.

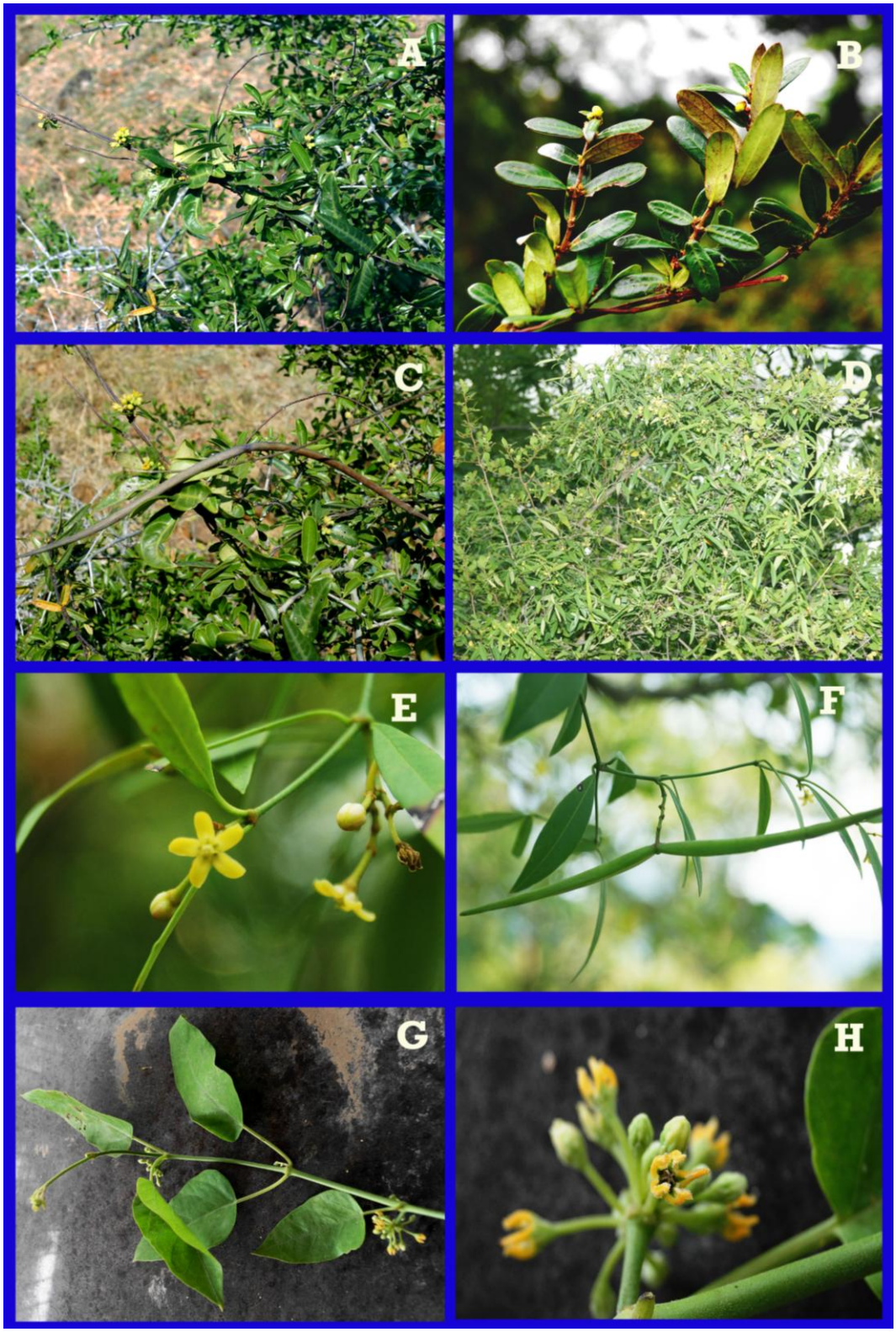

Fig. 2: A – *Hemidesmus indicus* (L.) R.Br. – Habit; B – *Hemidesmus indicus* (L.) R.Br. – A branch showing short leaves; C – *Hemidesmus indicus* (L.) R.Br. with flowers and fruits; D – *Secamone emetica* (Roxb.) R.Br. ex Schultes – Habit; E – *Secamone emetica* (Roxb.) R.Br. ex Schultes – Flowers; F – *Secamone emetica* (Roxb.) R.Br. ex Schultes – Follicles; G – *Leptadenia reticulata* (Retz.) Wight & Arn. – A branch with flowers; H – *Leptadenia reticulata* (Retz.) Wight & Arn. – Close view of flowers.

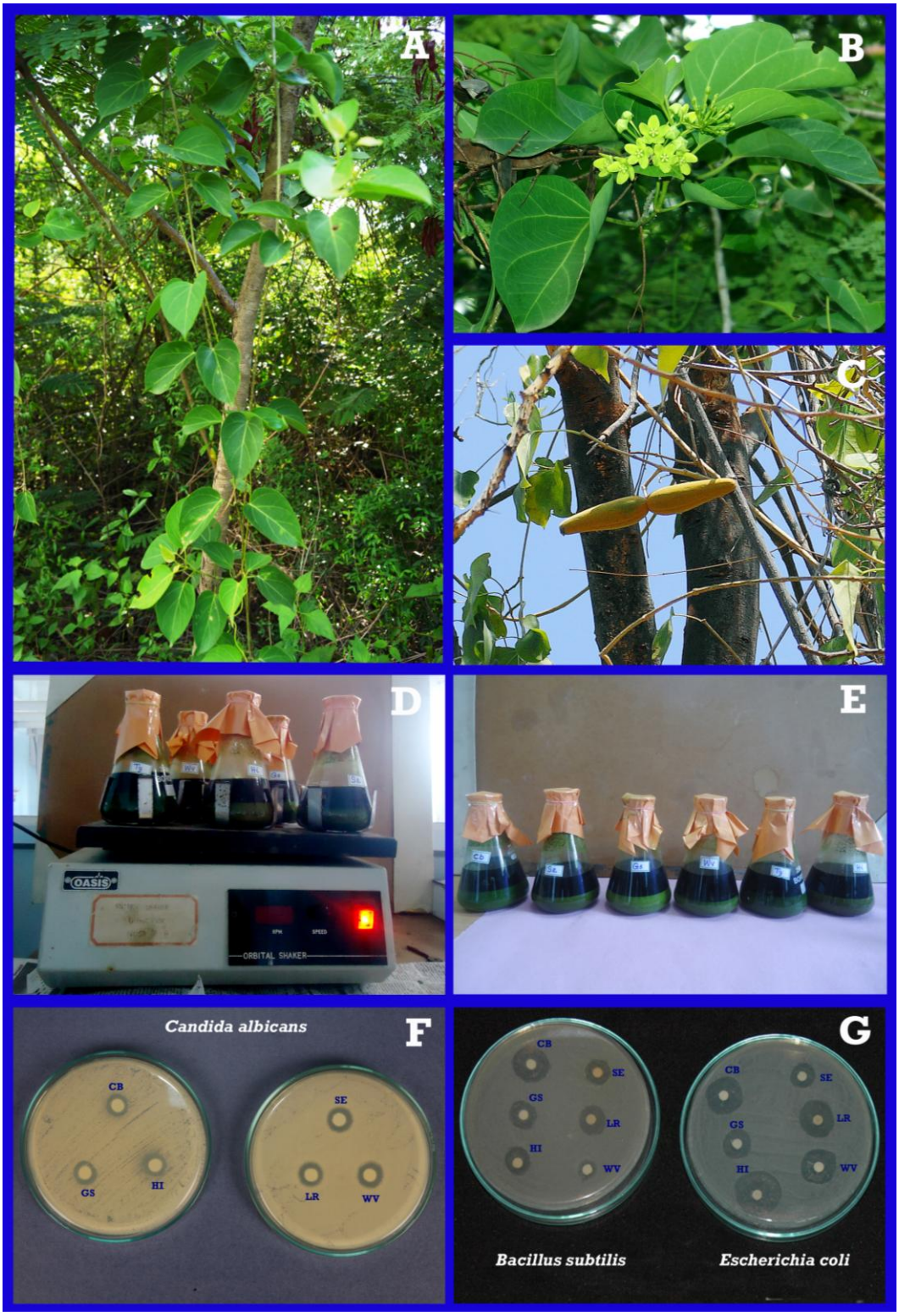

Fig. 3: A – *Wattakaka volubilis* (L.f) T. Cooke – Habit; B – *Wattakaka volubilis* (L.f) T. Cooke – Flowers; C – *Wattakaka volubilis* (L.f) T. Cooke – Follicles; D – Cold extraction flasks in Orbital Shaker; E – Extraction flasks after 48 hours; F – Antifungal activity of ethanolic leaf extracts against *Candida albicans*; G – Antibacterial activity of ethanolic leaf extracts against *Bacillus subtilis* and *Escherichia coli*; CB - *Cryptolepis buchanani*; GS- *Gymnema sylvestre*; HI - *Hemidesmus indicus*; E - *Secamone emetica*; LR - *Leptadenia reticulata*; WV - *Wattakaka volubilis*;

Table 1. Effect of antibiotic reference standards on test organisms.

1.	Antibiotic reference standard	Amphotericin	Ciprofloxacin
2.	Concentration (µg/disc)	30 µg	30 µg
3.	Test organisms	*Candida albicans*	*Bacillus subtilis* and *Escherichia coli*
4.	Zone of inhibition (cm)	2.5*	3.0*

* mean of three replicates.

Table 2: Antibacterial activity recorded in the ethanolic leaf extracts of selected ethnomedicinal Asclepiads against *Bacillus subtilis* and *Escherichia coli* (24 hrs; Reference standard: Ciprofloxacin).

Sl. No.	Ethanolic extract of the plant leaves used	Zone of inhibition (cm)*	
		Bacillus subtilis	*Escherichia coli*
1.	*Cryptolepis buchanani*	2.3	2.5
2.	*Gymnema sylvestre*	1.9	1.2
3.	*Hemidesmus indicus*	1.5	2.8
4.	*Secamone emetica*	1.6	1.4
5.	*Leptadenia reticulata*	1.7	2.0
6.	*Wattakaka volubilis*	0.9	2.1

* Values are mean of three replicates.

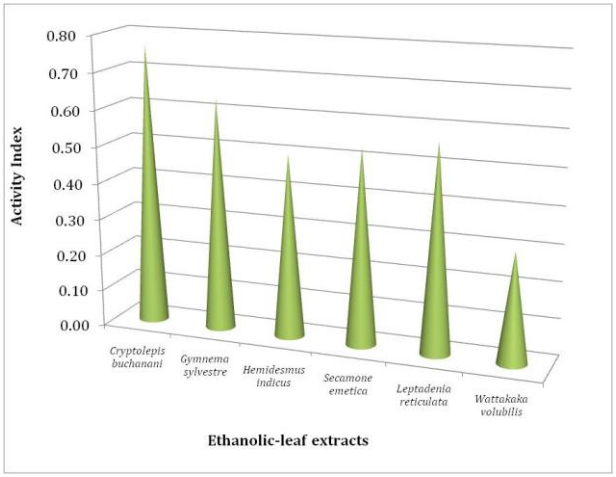

Fig. 4: Antibacterial activity index recorded in the ethanolic leaf extracts of selected ethnomedicinal Asclepiads against *Bacillus subtilis.* (24 hrs; Reference standard: Ciprofloxacin).

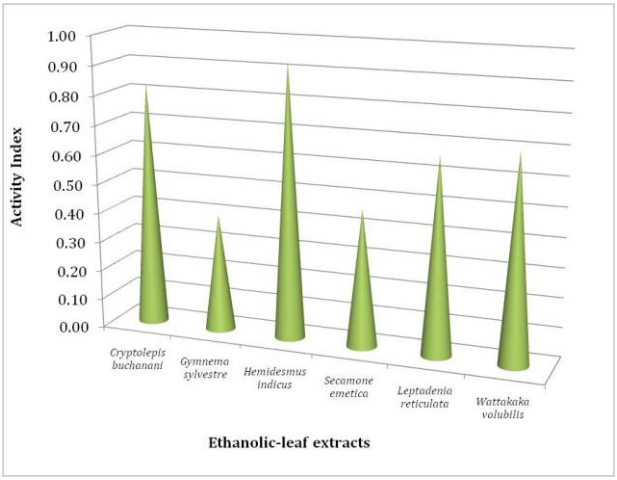

Fig. 5: Antibacterial activity index recorded in the ethanolic leaf extracts of selected ethnomedicinal Asclepiads against *Escherichia coli* (24 hrs; Reference standard: Ciprofloxacin).

Antibacterial activity index for the extracts of ethnomedicinal Asclepiads against *Bacillus subtilis* is provided in Fig. 4. The leaf extracts of *Hemidesmus indicus* exhibited a maximum inhibitory activity against *Escherichia coli* with a zone of inhibition of 2.8 cm, and this was followed by *Cryptolepis buchanani* with a zone of inhibition of 2.5 cm. The least antibacterial activity was observed for *Gymnema sylvestre* leaf extracts against *Escherichia coli* (1.2 cm).

The ethanolic extracts of the Asclepiads, *Wattakaka volubilis* (2.1 cm), *Leptadenia reticulata* (2.0 cm) and *Secamone emetica* (1.4 cm) showed moderate antibacterial activity against *Escherichia coli* (Table 2, Fig. 3G). Antibacterial activity index for the extracts of ethnomedicinal Asclepiads against *Escherichia coli* is provided in Fig. 5. The maximum antimicrobial activity reported in the current study fall in line with the study conducted by Gayathri and Kannabiran (2009). The results of

their study concludes that the aqueous extracts of *Hemidesmus indicus*, *Ficus benghalensis* and *Pterocarpus marspium* has significant antibacterial activity against different pathogenic bacteria, *Stahylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*.

Table 3. Antifungal activity recorded in the ethanolic leaf extracts of selected ethnomedicinal Asclepiads against *Candida albicans* (24 hrs; Reference standard: Amphotericin).

Sl. No.	Ethanolic extract of the plant leaves used	Zone of inhibition (cm)*
1.	*Cryptolepis buchanani*	1.2
2.	*Gymnema sylvestre*	1.1
3.	*Hemidesmus indicus*	1.3
4.	*Secamone emetica*	1.2
5.	*Leptadenia reticulata*	1.4
6.	*Wattakaka volubilis*	0.9

* Values are mean of three replicates.

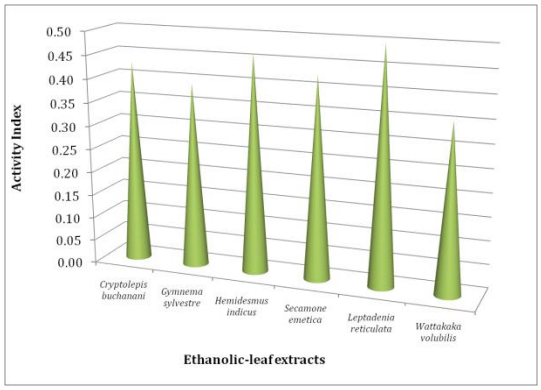

Fig. 6: Antifungal activity index recorded in the ethanolic leaf extracts of selected ethnomedicinal Asclepiads against *Candida albicans* (24 hrs; Reference standard: Amphotericin).

The highest zone of inhibition in *Leptadenia reticulata* leaf extracts was 1.4 cm against the fungus, *Candida albicans* followed by *Hemidesmus indicus* (1.3 cm), *Cryptolepis buchanani* (1.2 cm) =*Secamone emetica* (1.2 cm)>*Gymnema sylvestre* (1.1 cm) and the least zone of inhibition was by *Wattakaka* volubilis which showed a zone of inhibition of 0.9 cm (Table 3, Fig. 3F). Antifungal activity index for the extracts of ethnomedicinal Asclepiads against *Candida albicans* is provided in Fig. 2 which reflects the comparative level of activity with the standard antibiotics. The higher antifungal activity may be due to the bioactive compounds present in Asclepiadaceae members which played major role in various biological activities (Krishna Reddy et al., 2009; Joseph et al., 2011; Khatoon et al., 2013). All these studies suggest that the leaf extracts of ethnomedicinally important Asclepiads exert antimicrobial activity against selective bacteria and fungus that may be useful for the development of a new and effective antibacterial drug.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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