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Comparative phytochemical screening and antimicrobial efficacy studies on two endemic species - *Jatropha maheshwarii* Subr. & Nayar and *Jatropha villosa* Wight.

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Abstract
Jatropha maheshwarii Subr. & Nayar and *Jatropha villosa* Wight, belonging to the spurge family, are highly endemic species showing a narrow range of distribution. Traditionally the plants bear more folkloric usages as utilized by the rural communities. However, these species remains unexplored and underutilized. With this view in mind, the present investigation aims to evaluate the phytochemical constituents as well as comparative antimicrobial properties of both the species against various human bacterial and fungal pathogens. Antimicrobial efficacy was performed by disc diffusion method against the bacterial pathogens viz., *Staphylococcus aureus, Bacillus cereus, E. coli, Klebsiella pneumonia* and *Enterococcus faecalis* incubated for 24 hrs at 37 °C as well as the isolates of fungal pathogens *Candida albicans, Candida tropicalis, Candida parapsilosis, Candida cruzi* and *Aspergillus niger*. All the extracts of the plant viz., acetone, distilled water, methanol, chloroform, petroleum ether and benzene, studied in the present investigation exhibited varying degree of inhibitory effect against the selected human pathogens. It is authenticated that the plants revealed the presence of valuable phytochemicals and exhibited good antimicrobial activity, which supports its folkloric use and thus provides clue for its successful utility as a medicine.

Keywords: Antibacterial, Antifungal, *Jatropha maheshwarii*, *Jatropha villosa*, Phytochemical, Plant extracts.

1. Introduction
Euphorbiaceae “the spurge family” comprises 335 genera with 8000 species world over and most of them are ornamental, medicinal and energy crops. The genus *Jatropha* belongs to the tribe Joannesieae of Crotonoideae in the Euphorbiaceae family is a morphologically diverse genus encompassing 170 species distributed in almost all the tropical and subtropical regions of the world [1]. In India, the genus is reported with nine species [2]. “Jatros” is a Greek word means doctor and “Trophe” means nutrition. Most of the *Jatropha* species are native to new world and no complete review of the 66 old world *Jatropha* exists. Plants of this genus are herbs, shrubs or trees, monoecious (rarely dioecious), exudates are watery to white; possess poisonous substance in the sap/seed. Many species belonging to this family have been used traditionally for their medicinal properties. Two species viz. *Jatropha maheshwarii* and *Jatropha villosa* were chosen for the study. Among the various species under this genus, these two species are highly endemic in their distribution.
Jatropha maheshwarii Subr. and Nayar is an endemic plant commonly called as ‘Vel-athalai’ in Tamil. It is an evergreen under shrub, thick stem and dark green, petiole glabrous, leaves lanceolate. It is a fertile, drought hardy and rhizomatous plant having 22 chromosomes. Naturally its distribution is restricted only to the southern coastal belts of Kanyakumari, Thoothukudi and Tirunelveli districts of Tamil Nadu. This plant is notable for its wide usage by the locals against rheumatism, eczema, ringworms and as an insecticide. The light green viscid latex oozing from the plant parts is reported to have potential to arrest haemorrhage from eczema [3]. Traditionally, the extract obtained by boiling fresh leaves of *J. maheshwarii* with rice stool is used to treat inflammations and possess anti-inflammatory activity. Also fresh latex obtained from the plant is applied directly to treat mouth ulcers (Personal communication). Fresh tender stems are utilized as tooth brush by the local community. Further methanolic stem extract is reported to possess antimicrobial property against *Staphylococcus aureus* [4].

Jatropha villosa Wight. is known as 'thanakku' in Tamil. It is an endemic undershrub confined to Coimbatore, Dharmapuri, Salem and Nilgiris of Tamilnadu. The plant possesses 22 chromosomes [5]. Shoots are rusty – villow, leaves 4–6 inch in diameter, tomentose beneath, shallowly 3–5 lobes, 5 nerved. Cymes corymbose, peduncle stout, flowers pale yellow. Calyx lobes are lanceolate and corolla salver shaped. Stamens 8, basally connate, capsule 3 cm long [6, 7].

From the previous literature it was understood that these medicinal plants are unexplored, hence a detailed phytochemical and antimicrobial efficacy study was carried out. In this study, we aimed to evaluate the phytochemical constituents and to detect a possible inhibitory effect of different extracts of *Jatropha maheshwarii* and *Jatropha villosa* against the growth of various selected bacterial and fungal human pathogens tested by using agar disc diffusion method.

2. Material and Methods

2.1. Collection and authentication of plant material

To study the antimicrobial activity, disease free whole plants of *Jatropha maheshwarii* were freshly collected from coastal areas of Kanyakumari (08° 04.763' N & 077° 32.278' E) and *Jatropha villosa* from Coimbatore, Tamilnadu (10° 54.012' N & 076° 50.636' E), (Plate -1). The plants were identified and voucher specimens were deposited in the Herbarium, Department of Botany, Nesamony Memorial Christian College, Marthandam. Each of the samples were washed 3-4 times with running tap water followed by sterile distilled water and then shade dried to constant weight and ground to a coarse powder form using a domestic mixer and stored for further use.

2.2. Preparation of crude extracts

About 100 g of each powdered samples were weighed and taken separately for the respective species and were extracted with different solvents viz. acetone, distilled water, methanol, chloroform, petroleum ether and benzene individually using soxhlet's apparatus. The organic extracts obtained were evaporated to dryness by kept open in room temperature. On the other hand in case of aqueous extraction, the extract was evaporated to dryness at 50 °C in a water bath. This concentrate was later subjected to phytochemical analysis and microbial bioassays.

2.3. Phytochemical screening

The crude extracts of *J. maheshwarii* and *J. villosa* were subjected to phytochemical tests for plant secondary metabolites. Proteins were estimated by Lowry method [8] and amino acids by Ninhydrin test. Carbohydrates are estimated by Anthrone method [9]. Presence of fat or lipids was determined by solubility test. Flavanoids was estimated as method suggested by [10]. Steroids by Salkowski test. Alkaloids and tannins were estimated by the proposed methodology [11]. Presence of saponin was determined by the method suggested by [12, 13] and phenol by Folin Ciocalteu Reagent Method.

2.4. Antibacterial assay

Five human pathogenic bacterial strains were used for the present study i.e., *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Klebsiella pneumoniae* and *Enterococcus faecalis* were collected from Scudder laboratory, Nagercoil. A colony of each bacterium from the pure culture was transferred into liquid broth (Nutrient broth) using inoculation needle and incubated for about 24 h at 37 °C, maintained in axenic condition. The antibacterial activity was tested by agar disc diffusion method using Mueller Hinton Agar medium (MHA). The standard bacterial suspension was inoculated onto the surface of Mueller Hinton Agar plates. Using a sterile swab, the inoculums were swabbed over the entire surface of the agar plate, with the plate rotated approximately 60°A each time to ensure even distribution of the inoculum. Now the extracts obtained from both selected species were loaded on sterile discs (5 mm) separately with 50 µl of each extract followed by placing it on the surface of medium and the extract was allowed to diffuse. The plates were incubated at 37 ºC for 24 hrs. Along with this 30 µl Amikacin disc was studied for antibacterial activity as a positive control. The petriplates were checked for inhibition zone formation. The inhibition zone obtained around the discs was measured using a transparent ruler in millimeter. The study was performed in duplicates.

2.5. Fungal Isolates and Bioassay

Isolates of fungal pathogens *Candida albicans, Candida tropicalis, Candida parapsilosis, Candida cruzi* and *Aspergillus niger* were obtained from Scudder laboratory, Nagercoil. The cultures were maintained on potato dextrose agar until further study. Aliquots of potato dextrose agar medium were poured in sterile petridishes. The plates were allowed to solidify for 5 minutes and 0.1% inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 minutes. The crude extract (20 µl) was loaded on 4 mm sterile disc. The loaded discs were placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were incubated for 72 hours at 28± 2 ^{0}C. Observations were made on the growth of fungal mycelium as influenced by the plant extracts. Based on the growth rate of fungi in response to plant extract, the rate of inhibition was measured in millimeter. The study was performed in duplicates.

3. Results and Discussion

Various phytochemical constituents of selected plant species analysed were summarized in Table - 5. From the results it is revealed that, *J. maheshwarii* shows the presence of phenol, coumarins, alkaloids, flavanoids, quinones, steroids, amino acids, saponins, xanthoproteins, tannins, carboxylic acids and carbohydrates. Presence of alkaloid, tannin, flavonoid, steroid, glycoside, saponins and coumarins were earlier reported by [14] while our findings further supplements the presence of phenol, quinines, aminoacids, xanthoproteins, carboxylic acid and carbohydrates. *Jatropha villosa* shown positive results with the presence of proteins, phenol, coumarins, alkaloids, flavanoids, quinines, steroids, amino acids, saponins, xanthoproteins, tannins, carboxylic acids and carbohydrates. Similar phytochemicals like saponins, steroids, alkaloids, phenolic groups and flavonoids were also reported in *J. curcas* [15]. Further, the presence of saponin, tannins, phenols, cellulose, nitrogen, crude protein, calcium, phosphorus and potassium were also reported in *J. gossypifolia* [16].

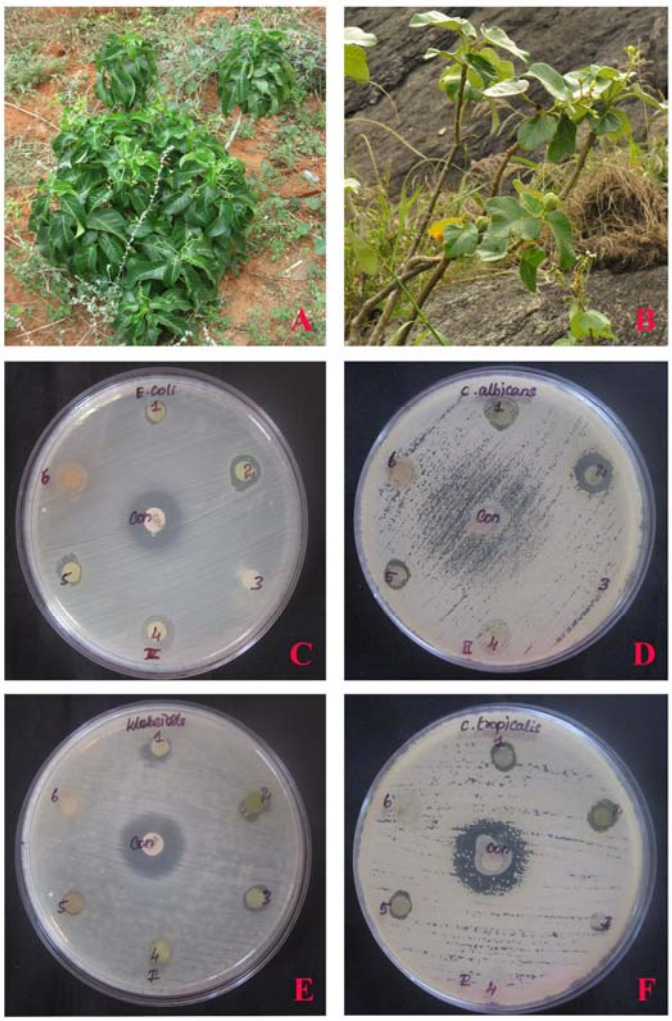

Plate 1 - A. Habit of *J. maheshwarii* **B**. Habit of *J. villosa*
C – F. Antimicrobial activity of benzene, acetone, chloroform, petroleum ether, methanol and distilled water extracts of *J. maheshwarii* on: **C** - *Escherichia coli*; **D** - *Candida albicans* & *J. villosa* on: **E** - *Klebsiella pneumonia*; **F** - *Candida tropicalis*.

Comparative antimicrobial study of various plant extracts of *J. maheshwarii* and *J. villosa* showed varied degree of zone of inhibition against the tested bacterial and fungal pathogens. The acetone, distilled water, methanol, chloroform, petroleum ether and benzene extracts extracted from the plant parts of *J. maheshwarii* showed antibacterial activity as reported in Table - 1. Here, the reference drug gentamycin investigated exhibited higher antimicrobial activity against all the test isolates except *E. faecalis*. Among the bacterial isolates investigated, the acetone extract exhibited the maximum activity against *E. coli* (10 mm) and *K. pneumoniae* (8 mm). Followed this benzene extract had the activity against *K. pneumoniae* (8 mm). However, the methanol extract showed moderate amount of antimicrobial activity only against *E. coli* with 7mm inhibition zone formation. Similar results were also reported in *J. curcas*[17]. Further this result was quite contrary with an earlier report, where best activity on methanolic stem extract of *J. maheshwarii* against *S. aureus* was reported [4]. It is assumed that all these antibacterial activities exhibited may be due to the presence of compounds like phytol, 1, 2-benzenedicarboxylic acid, diisooctyl ester, squalene etc. which posses high antimicrobial properties as reported in *J. maheshwarii* [3]. All other extracts studied were found ineffective, where it doesn't exhibit zone formation.

The acetone, distilled water, methanol, chloroform, petroleum ether and benzene extracts extracted from the plant parts of *J. villosa* showed antibacterial activity as reported in Table - 2. The effects of the crude extracts of *J. villosa* on bacterial isolates were compared favourably with a standard antibiotic (Gentamycin) and it was found that all the isolates possessed a highest activity except *E. faecalis*. The result obtained indicates that the acetone extract (10 mm) and methanol extract (9 mm) exhibited the most significant activity against *E. coli.* These results were in corroborative with the findings in *J. curcas* [15, 18]. Followed this, chloroform extract exhibited moderate activity with 7mm zone formation against *E. coli* and *Klebsiella pneumonia*. Related results were also reported in *J. gossypifolia* [16]. Also Benzene extract too exhibited moderate activity with 7mm zone formation against *E. coli*. Similar results were reported in the leaf extract of *J. curcas* [19], *J. gossypifolia* [20]. In the case of *K. pneumoniae*, benzene and acetone extracts posses activity with 8 mm zone formation and chloroform extract with 7 mm zone formation. Similar results were reported in aqueous and methanolic extracts of *J curcas* [21]. Petroleum ether extract alone shown positive response against *S. aureus* (8 mm). Relative findings were reported in hexane extract of *J. podagrica* [22]. While with *B. cereus*, the benzene and petroleum ether extracts exhibited activity with 8 mm and 9mm zone formation respectively. Further *E. faecalis* fails to posses activity against extracts investigated.

The results of antifungal assay tested against *Candida albicans, Candida tropicalis, Candida parapsilosis, Candida*

cruzi and *Aspergillus niger* using crude extracts obtained from the aerial parts of *J. maheshwarii* by using acetone, distilled water, methanol, chloroform, petroleum ether and benzene are shown in Table - 3. The result reveals that acetone extract treated exposed better activity against all the fungal pathogens tested, with the zone formation of 11 mm, 13 mm, 11 mm, 10 mm and 10 mm respectively. Benzene extract also exhibited activity against all the fungal pathogens except *Aspergillus niger* with the zone formation of 10 mm, 8 mm, 9 mm and 9 mm respectively. Further methanol extract bared activity with *C. albicans* and *C. cruzi* by the formation of inhibitory zones of 8mm each. Similar results were reported with the hexane extract of *J. curcas* [23]. The standard antibiotic flucanazole was tested as the control against all the pathogens selected for the study, which possess highest activity with *C. tropicalis* (20 mm), *C. parapsilosis* (18 mm) and no activity with other pathogens.

Candida albicans, Candida tropicalis, Candida parapsilosis, Candida cruzi and *Aspergillus niger* were also subjected to antifungal assay using crude extracts obtained from the aerial parts of *J. villosa* by using acetone, distilled water, methanol, chloroform, petroleum ether and benzene as shown in Table - 4. From the results it is evident that acetone extract possessed the maximum activity against *Candida albicans* (10 mm), *Candida tropicalis* (10 mm) and *Candida parapsilosis* (9 mm). These results were in corroborative with the findings reported from the latex of *J. curcas* [24]. Methanol extract in addition exhibited activity against *Candida albicans* (10mm), *Candida tropicalis* (7 mm) and *Candida cruzi* (7 mm). Comparable results were reported in the stem bark extract of *J. curcas* [17]. Moreover benzene extract shown positive response against *Candida tropicalis* with the zone formation of 9mm. The control (fluconazole) tested possessed activity only with *Candida tropicalis* (20 mm) and was ineffective with rest of the pathogens.

Testing of the plant extracts obtained from *J. maheshwarii* and *J. villosa* for antimicrobial activity showed varying degrees of performance against various bacteria (*Staphylococcus aureus, Bacillus cereus, E. coli, Klebsiella pneumonia* and *Enterococcus faecalis*) and fungi (*Candida albicans, Candida tropicalis, Candida parapsilosis, Candida cruzi* and *Aspergillus niger*). The activity against these pathogens might be due to the presence various phytochemical constituents in them. Mostly pharmacological activity of medicinal plant residues is due to so called secondary metabolites, since they are comparatively smaller molecules in contrast to the primary metabolites such as proteins, carbohydrates and lipids [25]. In the present study, it is observed that, growth of bacterial as well as fungal colonies has been arrested or controlled by various extracts used. Recently a number of plants have been reported for their antimicrobial properties across the globe [26 – 30].

Perusal of the previous literature of two species investigated reveals that, reports are so meager with *J. maheshwarii* only by the existence of few studies on GCMS analysis [3], pharmacognostical [14] and antimicrobial activity [4]. *Jatropha villosa* remains an unexploited and underutilized species. There exists no report on its phytochemical as well as antimicrobial activity so far. Thus the present study shows the presence of antimicrobial activity in *J. villosa* for the first time.

Table 1: Antibacterial assay of *Jatropha maheshwarii.*

Micro Organisms	Plant Extracts						
	Benzene (mm) 1	Acetone (mm) 2	Chloroform (mm) 3	Petroleum Ether (mm) 4	Methanol (mm) 5	Distilled water (mm) 6	Control Amikacin (mm)
Staphylococcus aureus	-	-	-	-	-	-	21
Bacillus cereus	-	-	-	-	-	-	21
Escherichia coli	-	10	-	-	7	-	17
Klebsiella pneumoniae	8	8	-	-	-	-	18
Enterococcus faecalis	-	-	-	-	-	-	-

Table 2: Antibacterial assay of *Jatropha villosa*

Micro Organisms	Plant Extracts						
	Benzene (mm) 1	Acetone (mm) 2	Chloroform (mm) 3	Petroleum ether (mm) 4	Methanol (mm) 5	Distilled water (mm) 6	Control Amikacin (mm)
Staphylococcus aureus	-	-	-	**8**	-	-	**22**
Bacillus cereus	**8**	-	-	**9**	-	-	**22**
Escherichia coli	**7**	**10**	**7**	-	**9**	-	**15**
Klebsiella pneumoniae	**8**	**8**	**7**	-	-	-	**19**
Enterococcus faecalis	-	-	-	-	-	-	-

Table 3: Antifungal assay of *Jatropha maheshwarii*

Micro Organisms	Plant Extracts						
	Benzene (mm) 1	Acetone (mm) 2	Chloroform (mm) 3	Petroleum ether (mm) 4	Methanol (mm) 5	Distilled water (mm) 6	Control Flucanazole (mm)
Candida albicans	10	11	-	-	8	-	-
Candida tropicalis	8	13	-	-	-	-	20
Candida parapsilosis	9	11	-	-	-	-	18
Candida cruzi	9	10	-	-	8	-	-
Aspergillus niger	-	10	-	-	-	-	-

Table 4: Antifungal assay of *Jatropha villosa*

Micro Organisms	Plant Extracts						
	Benzene (mm) 1	Acetone (mm) 2	Chloroform (mm) 3	Petroleum ether (mm) 4	Methanol (mm) 5	Distilled water (mm) 6	Control Flucanazole (mm)
Candida albicans	-	10	-	-	10	-	-
Candida tropicalis	9	10	-	-	7	-	20
Candida parapsilosis	-	9	-	-	-	-	-
Candida cruzi	-	-	-	-	7	-	-
Aspergillus niger	-	-	-	-	-	-	-

Table 5: Phytochemical constituents of *Jatropha maheshwarii* and *Jatropha villosa*

Sl. No.	Plant	Extracts	Phytochemicals												
			Phenol	Coumarins	Alkaloids	Flavanoids	Quinones	Steroids	Proteins	Amino acids	Saponins	Xanthoproteins	Tannin	Carboxylic acids	Carbohydrates
1	*Jatropha maheshwarii*	Dis. Water	+	-	-	-	+	-	-	+	-	-	+	-	-
		Pet. Ether	+	+	+	+	-	-	-	+	+	+	-	-	-
		Acetone	-	-	-	-	-	+	-	-	+	+	+	-	+
		Chloroform	-	+	-	+	-	-	-	+	-	-	-	+	-
		Benzene	-	-	-	-	+	-	-	-	-	-	-	-	+
2	*Jatropha villosa*	Dis. Water	+	-	+	-	+	-	+	-	-	-	+	-	+
		Pet. Ether	-	-	-	-	-	-	+	-	+	+	-	-	+
		Acetone	-	-	-	-	-	+	+	+	+	+	-	-	-
		Chloroform	-	+	-	+	-	-	-	-	-	-	-	-	-
		Benzene	+	-	-	-	+	-	-	-	-	-	-	+	-

4. Conclusion

Both of the plant species investigated is highly endemic in their distribution with limited populations. They encompass a plenty of folkloric usages exhibited by various traditional communities. The present study has revealed and confirmed that the investigated plant extracts could be used for the treatment of various infections caused by the selected pathogens. The presence of antimicrobial properties further provides clues for the identification of phytochemical compounds from them. Further it supports the folkloric usage of these plant species and suggested that it possess compounds with antimicrobial properties and hence shows that *J. maheshwarii* and *J. villosa* could be exploited as source for new potent antibiotic in the future generation.

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