

Antimicrobial Efficacy Studies on Rhizome and Fruit pulp Extracts of a Steno Endemic species – *Jatropha maheshwarii* Subr. & Nayar

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Abstract

Rhizome, fruits and seeds of plants constitute a major role in traditional medicine by encompassing therapeutically valuable phytochemicals. Here, the medicinal importance and bioefficacy status of rhizome and fruit pulp extracts of *Jatropha maheshwarii* Subr. & Nayar against selected human pathogenic microbes were examined. Ethyl alcohol, benzene, chloroform, hexane, acetone and distilled water extracts of the rhizome and fruit pulp samples were screened against selected bacterial strains viz. *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterobacter aerogenes* as well as fungal strains viz. *Aspergillus niger* and *Candida albicans* using agar well diffusion method.

Keywords: Antibacterial, Antifungal, *Jatropha maheshwarii*, Agar well diffusion, Fruit pulp and Rhizome.

Introduction

The genus *Jatropha* belongs to the family, Euphorbiaceae and is distributed in India with 13 species [1]. The name *Jatropha* is derived from the Greek words *Jatros* (doctor) and *Trophe* (nutrition). Many species belonging to this family have been used traditionally for their medicinal properties. Among this, *Jatropha maheshwarii* was chosen for the study. Among various species under this genus, *Jatropha maheshwarii* Subr. and Nayar is an endemic species whose distribution is constrained to the southern coastal belts, plains and hilly regions of Kanyakumari, Thoothukudi and Tirunelveli districts of Tamil Nadu, extending to the west coast up to Thiruvananthapuram district of Kerala [1]. This plant is commonly called as 'Athalai' [2], 'Vel-athalai' [3], and 'Kattamannaku' [4] in Tamil. It is an evergreen under shrub, thick stem and dark green, petiole glabrous, leaves ovate-lanceolate [5]. It is a fertile, drought hardy and rhizomatous plant which attains a height of about 2m having 22 chromosomes [6]. This plant is notable for its valuable traditional medicinal properties among the locals against rheumatism, eczema, ringworms and as an insecticide [4], [7]. The latex obtained from the plant parts is reported to have potential to arrest hemorrhage from eczema and also to treat mouth ulcers. The leaf extract is reported to treat inflammations and possess anti-inflammatory activity [3]. Fresh tender stems are utilized as tooth brush by the local community. Further antibacterial effect of methanolic stem extract is reported against *Staphylococcus aureus* [8]. Similarly the effect of crude extracts against selected bacterial and fungal pathogens were also reported [3].

In this study, we aimed to detect a possible inhibitory effect of different extracts of rhizome and fruit pulp of *Jatropha maheshwarii* on the growth of various selected human bacterial and fungal pathogens tested by using agar well diffusion method.

Materials and Methods

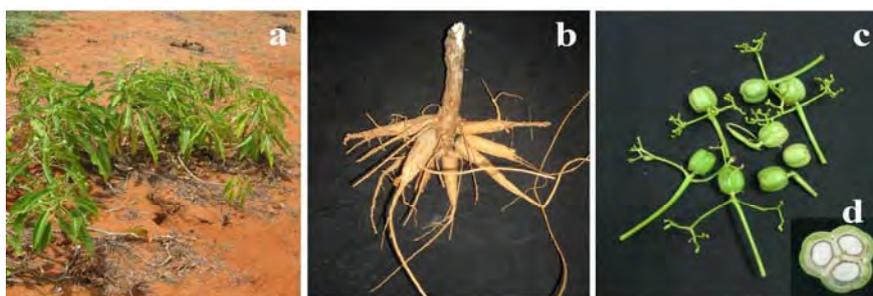


Fig. 1. *Jatropha maheshwarii* (a) Habit (b) Rhizome (c) Fruits (d) C.S of fruit showing fruit pulp

Material collection:

The rhizome and fruits of *Jatropha maheshwarii* were freshly collected near the coastal areas of Thoothukudi (08 45.818' N & 078 04.489' E), (Fig. 1). The plant parts were identified and voucher specimen of the plant was deposited in the Herbarium, Department of Botany, Nesamony Memorial Christian College, Marthandam. The samples were washed in running tap water followed by sterile distilled water. They were separated, shade dried and grinded into fine powder form using a domestic mixer and stored for further use.

Sample extraction:

20g of dry powdered samples were weighed and taken separately and were subjected to extraction with different solvents viz. ethyl alcohol, benzene, chloroform, hexane, acetone and distilled water individually using soxhlet's apparatus. The extracts obtained were pooled and evaporated in air at room temperature to obtain a final residue. While for aqueous extraction, the extract was evaporated to dryness by heating in a water bath. The concentrates obtained were refrigerated and later subjected to antimicrobial assays.

Antibacterial assay – Agar well diffusion method

Petriplates containing 20ml Muller Hinton medium were seeded with 3 hours grown culture of bacterial strains such as *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterobacter aerogenes*. Wells of approximately 10mm was bored using a well cutter and samples in 50 μ l conc. was added. The antimicrobials present in the plant extracts are allowed to diffuse out into the medium and interact with freshly seeded test organisms. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well in millimeters [9]. The study was performed in duplicates.

Antifungal assay – Agar well diffusion method

Aliquots of potato dextrose agar (PDA) medium were poured in sterile petridishes. The plates were allowed to solidify for 5 minutes and 0.1% inoculums suspension of *Candida albicans* and *Aspergillus niger* were swabbed uniformly and the inoculum was allowed to dry for 5 minutes. Wells of approximately 10mm was bored using a well cutter and samples in 50 μ l conc. were added. The compound was allowed to diffuse for 5 minutes and the plates were incubated for 72 hours at 28 ± 2 °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.

Results and Discussion**Antibacterial assay:**

The stock crude extracts prepared from the rhizome and fruit pulp samples of *J. maheshwarii* by using benzene, acetone, ethanol, chloroform, hexane and distilled water were subjected to antibacterial activity against *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterobacter aerogenes*, and the results were recorded (Table 1 & 2).

The observed results indicates that in the case of rhizomatous extracts of *Jatropha maheshwarii*, the reference drug gentamycin investigated exhibited higher antibacterial activity against all the test isolates. Most significant activity was reported with chloroform extract against *E. Coli* (19mm), *Enterococcus faecalis* (11mm), *Staphylococcus aureus* (11mm) and *Enterobacter aerogenes* (11mm). These results corroborate with the findings on chloroform leaf extract of *Murraya koenigii*[10]. Similar results were also reported in *Syzygium cumini* and *Adenanthera pavonina*[11]. Followed this, hexane and ethanol extracts exhibited best activity against *Enterococcus faecalis* (11mm), *Escherichia coli* (18mm), *Enterobacter aerogenes* (11mm) and *Enterococcus faecalis* (14mm), *Pseudomonas aeruginosa* (18mm), *Enterobacter aerogenes* (14mm) respectively. Comparative results were earlier reported with methanolic rhizome extract of *Rheum australe* [12], stem bark extract of *Jatropha podagrica*[13] and leaf extract of *J. curcas* [14]. Also relative findings on ethanolic and aqueous leaf extracts were noticed in *Tarenna asiatica*[15]. Distilled water extract has shown moderate activity against *Enterococcus faecalis* and *Enterobacter aerogenes* with 10mm and 13mm zone formation respectively. These results were in conformity with similar report on the rhizome extract of *Zingiber cassumunar*[16]. Acetone extract exhibited less activity only with *Staphylococcus aureus* (10mm). Similar least activity with acetone root extract was noticed in *Euphorbia resinifera*[17]. Further benzene extract doesn't exhibited activity against any of the pathogens investigated. The results obtained were quite contrary with an earlier report on *J. maheshwarii* [3], where acetone extract is reported to possess more activity and chloroform extract with no activity. While in the present investigation, rhizomatous acetone extract exhibited least activity and chloroform extract with most significant activity. This needs to be evaluated.

The effects of the fruit pulp extracts of *J. maheshwarii* on bacterial isolates were compared favourably with a standard antibiotic (Gentamycin) and it was found that all the isolates possessed a highest activity. Further, the results obtained revealed that acetone extract acquired significant results against all the pathogens tested which was authenticated by the zone formation of 21mm, 21mm, 23mm, 11mm and 21mm respectively.

Relative findings were reported with methanolic stem extract of *J. maheshwarii* against *S. aureus*[8]. Also the results substantiate the findings on seed coat extracts of *Cassia alata*[18], *Cajanus cajan*, *Vigna unguiculata*, *Vigna mungo*[19] and leaf extract of *Jatropha gossypifolia*[20]. Ethanol and benzene extracts exhibited moderate activity with zone formation against *Pseudomonas aeruginosa*, *Escherichia coli* and *Enterobacter aerogenes* (20mm, 16mm, 13mm and 11mm, 20mm, 20mm respectively). These results were supported by the reports on fruit peel extract of *Citrus karna*[21], fruit pericarp extract of *Jatropha curcas*[22] and root extract of *Jatropha glandulifera*[23]. All other extracts analysed reported negative results with no inhibition zone formation.

Antifungal assay:

The stock crude extracts prepared from the rhizome and fruit pulp samples of *J. maheshwarii* by using benzene, acetone, ethanol, chloroform, hexane and distilled water were subjected to antifungal activity against *Aspergillus niger* and *Candida albicans* and the results were recorded (Table 1 & 2).

The result shows that, the reference drug clotrimazole investigated exhibited higher antifungal activity against the test isolates. Further the rhizomatous ethanolic extract, alone possessed activity against *Candida albicans* and *Aspergillus niger* with the inhibition zone formation of 10mm respectively. This result agrees with the report on root extract of *Jatropha podagrica*[13], sap extract of *Jatropha multifida*[24] and plant extract of *Jatropha dioica*[25]. Also similar results were reported in *Murraya koenigii* against *C. utilis*[10]. All other extracts investigated exhibited negative results with no zone formation.

Similarly in the case of fruit pulp samples, the reference drug clotrimazole exhibited best activity. Followed this, ethanolic extract alone exhibited activity only with *Aspergillus niger* with 11mm zone formation. Comparative results were also reported with fruit pulp and whole fruit extracts of *Jatropha curcas*[26]. Meanwhile, the reference drug investigated exhibited significant activity against the test isolates in both the cases.

Conclusion

From the results obtained, it can be concluded that the rhizome and fruit pulp of *J. maheshwarii* would be a good source of medicine against various ailments caused by the human bacterial and fungal pathogens investigated. Traditional knowledge has greater utility value as it saves time, effort and expenditure in the commercialization of drugs. Traditionally these plant parts are enormously utilized by the local communities as a remedy against various diseases which infects humans as well as animals. Thus, the present study authenticates the usage of these plant parts as a good antimicrobial agent. However sophisticated research on the same is absolutely necessary for its successful utility to the mankind.

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Table 1. Antimicrobial assay of Rhizome extracts of *Jatropha maheshwarii*

Sl. No.	Microorganisms	Rhizome						
		Hexane	Chloroform	Acetone	Ethanol	Benzene	Dis. H ₂ O	Gentamycin (Control)
1.	<i>Enterococcus faecalis</i>	11mm	11mm	-	14mm	-	10mm	35mm
2.	<i>Staphylococcus aureus</i>	-	11mm	10mm	-	-	-	25mm
3.	<i>Pseudomonas aeruginosa</i>	-	-	-	18mm	-	-	34mm
4.	<i>Escherichia coli</i>	18mm	19mm	-	-	-	-	30mm
5.	<i>Enterobacter aerogenes</i>	11mm	11mm	-	14mm	-	13mm	25mm
Antifungal activity								Clotrimazole (control)
1.	<i>Candida albicans</i>	-	-	-	10mm	-	-	24mm
2.	<i>Aspergillus niger</i>	-	-	-	10mm	-	-	28mm

Table 2. Antimicrobial assay of Fruit pulp extracts of *Jatropha maheshwarii*

Sl. No.	Microorganisms	Seed coat						
		Hexane	Chloroform	Acetone	Ethanol	Benzene	Dis. H ₂ O	Gentamycin (Control)
1.	<i>Enterococcus faecalis</i>	-	-	21mm	-	-	-	35mm
2.	<i>Staphylococcus aureus</i>	-	-	21mm	-	-	-	25mm
3.	<i>Pseudomonas aeruginosa</i>	-	-	23mm	20mm	11mm	-	34mm
4.	<i>Escherichia coli</i>	-	-	11mm	16mm	20mm	-	30mm
5.	<i>Enterobacter aerogenes</i>	-	-	21mm	13mm	20mm	-	25mm
Antifungal activity								Clotrimazole (control)
1.	<i>Candida albicans</i>	-	-	-	-	-	-	24mm
2.	<i>Aspergillus niger</i>	-	-	-	11mm	-	-	28mm