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Research Article

Antimicrobial, Phytochemical and Insecticidal Properties of *Jatropha* Species and Wild *Ricinus communis* L. Found in Mauritius

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ABSTRACT

Jatropha species and *Ricinus communis* L. (Euphorbiaceae) are important medicinal plants growing in both tropical and warm temperate regions of Africa, Southern Asia and Malaysia and are widely distributed throughout the island of Mauritius. These medicinal plants have been reported in several research works and valued for their various uses in traditional medicine for curative properties against inflammation, rheumatism, respiratory disorders, fever, bacterial infection and jaundice, among others. Publications exist on the various traditional and ethno medical uses of the plants, however no known scientific studies have been undertaken locally using *Jatropha* species and *Ricinus communis*, and no toxicity against Bactrocera insects have been reported . In this context, the present study was carried out to evaluate the phytochemical, antimicrobial activities and insecticidal properties of different *Jatropha* species and *Ricinus communis* (castor). The disc diffusion, MIC and toxicity assays tested the antimicrobial sensitivity and activity of ten microorganisms, lethality effect of the crude solvent extracts.

Keywords: *Jatropha curcas*, *Ricinus communis*, antimicrobial, phytochemical, insectici.

INTRODUCTION

Plants and their derivatives have long been used as both drugs and dietary supplements by man. According to Mahajan[1] up to 50 % of current pharmaceutical products were derived from plants but none were used as antimicrobials. Plants are rich sources of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found to have *in vitro* antimicrobial properties[2]. The development of microbial antibiotic resistance has required global search for new antimicrobials of preferably plant origin. Euphorbiaceae, the spurge family, is a large family of flowering plants with about 300 genera and around 7,500 species[3]. The spurges are herbs, shrubs or trees[4]. The genus Jatropha belongs to the Euphorbiaceae family, tribe Joannesieae,that has about 170 species. The name Jatropha is derived from the Greek word '*jatros*' (doctor) and '*trophe*' (food)[5]. This family occurs mainly in the tropics. The species are from the Indo-Malayan region, tropical America and tropical Africa and some subtropical countries like South Africa, Madagascar and Mauritius[6]. Jatropha species are used in traditional folklore medicine to cure various ailments in Africa, Asia and Latin America[7]. The plants usage as traditional health remedies is popular in Asia, Latin America and Africa.

The island of Mauritius is situated in the Indian Ocean 2,000 km (1242 miles) off the south east coast of the African continent (latitude 20° 17' S and longitude 57 ° 33' E). Due to its volcanic origin, age, location and soil composition, Mauritius is home to a diversity of flora and fauna. Plants of the Euphorbiaceae family are widely cultivated in Mauritius and have been reported to possess traditional antiseptic properties[5] all around the world. It contains a wide range of phytochemicals to which its antimicrobial activity is often attributed to[8]. Mauritius being multi-ethnic and multi-cultural and with ancestors of African, European and Indian origin, traditional medicine has a profound root in the Mauritian society.

Jatropha species and *Ricinus communis* L. are non-edible multipurpose shrubs belonging to the family of Euphorbiaceae. They are an uncultivated, non-food wild-species which grow around the island in backyards and in the wild. Different parts of the plant are used locally as ethno medicine. Many studies have been done to demonstrate the efficacy of *Jatropha* species against a wide array of bacteria[9,10,11,12] and fungi[13]. Several studies have also revealed that J. curcas has anticancer and antitumor properties[14,15]. Moreover, phytochemical studies carried out on *Jatropha* have revealed the presence of secondary metabolites. However, none of the studies have compared leaves of four different species of *Jatropha* using two different solvents for extraction and testing their ability to hinder growth of bacteria and fungus, their phytochemical constituent and testing their insecticidal properties on larvae of *Bactrocera zonata* and *Bactrocera cucurbitae*. The larvae of these two fruit fly species are very adaptable to different climates and have the capacity to survive on different host plants such as peach, guava, mango, citrus, cucurbits and mango. They cause serious economic losses, either by direct damage to fruit or by insecticide treatments[16].

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(a) *(b)* *(c)* *(d)* *(e)*

Fig. 1: Mature leaf samples of the selective medicinal plants
(a) J. multifida L.
(b) Ricinus communis L.
(c) J.curcas L.
(d) J. podagrica Hook.
(e.) J. integerrima Jack.

Table 1: Preliminary screening of phytochemicals in the two solvent crude extracts of *Jatropha* species and *Ricinus communis* using Test-tubes and TLC methods

Plants	Phytochemical					
	Alkaloids	Coumarins	Flavanoids	Steroids	Tannins	Phenols
J.curcas			(R_f value)			(R_f value)
Ethyl acetate	++	++	++(0.86)	--	--	--
Methanol	++	--	++(0.87)	++	++	++(0.70)
J. integerrima						
Ethyl acetate	++	--	++(0.73)	++	++	++(0.62)
Methanol	++	--	++(0.74)	++	--	--
J. podagrica						
Ethyl acetate	++	--	++(0.51)	++	++	--
Methanol	++	++	++(0.55)	--	++	++(0.75)
J. multifida						
Ethyl acetate	++	--	--	++	++	++(0.74)
Methanol	++	--	++(0.42)	++	--	++(0.72)
Ricinus communis						
Ethyl acetate	++	++	++(0.68)	--	--	++(0.69)
Methanol	++	--	++(0.69)	--	++	--

(++) Presence; (--) Absence; R_f: Retention factor

MATERIAL AND METHODS

Plant Materials: Mature leaf samples (Figure 1.0) were collected from different localities of Mauritius in Curepipe (Central Plateau of Mauritius), Nouvelle France (South of Mauritius) and Grand Baie (North of Mauritius). A small sample of the each plant specimen was sent to the Mauritius Herbarium, Réduit, for identification. The fresh plant materials were cleaned, washed under running tap water, air-dried for three to four days, then were coarsely pounded and stored in tightly closed plastic containers at 4ºC.

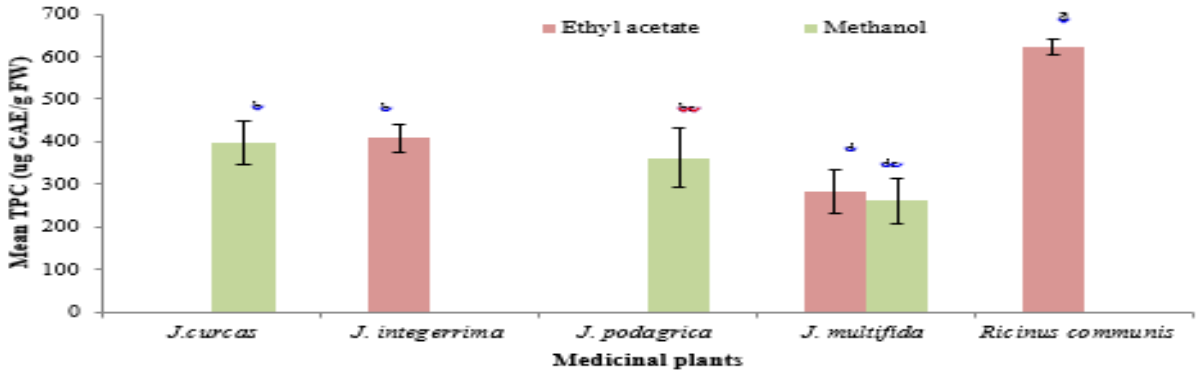
Chemicals and materials: All the chemicals and Pre-coated silica gel 60 F254 (TLC), were purchased from Sigma Chemicals®; the media, standard antibiotics from Hi-Media®; and Oxoid® and microplates from Merck®. The solvents used for extraction were of analytical reagent grade (AR).

Metabolite extraction: The decoction method was used to obtain the plant crude extracts. 20 g of fresh leaves were allowed to be macerated in 40 mL of two different solvent systems: methanol and ethyl acetate for 48 hours. The crude extracts were filtered using Whatman filter paper, pore size 15-18 µm, allowed to concentrate in a ventilated fume cupboard at room temperature and then stored at 4ºC. in the dark bottles for further use.

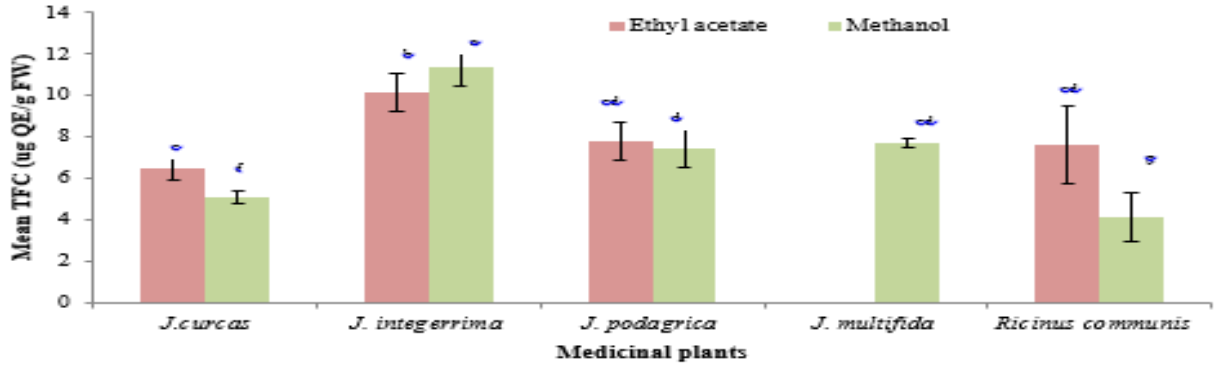
Phytochemical screening

Qualitative tests: The tests to detect the presence of flavonoids, alkaloids, saponins, steroids, tannins, coumarins and phenols were carried out according to the method described by Harborne[17]. All screenings were based on a series of test tube tests. The crude solvent extracts were used to test Phenols and alkaloids were also determined by thin layer chromatography (TLC) and UV techniques[18,19].

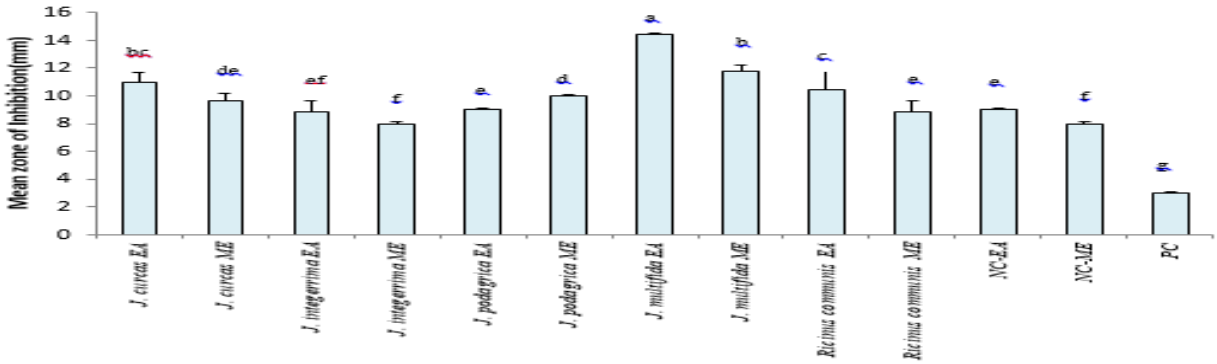
Quantitative determination of Total Flavonoid and Phenol content: The total flavonoid content was measured by AlCl3 (Aluminium chloride) colorimetric assay. To 500 µL of extract and 2000 µL of distilled water, 5% of sodium



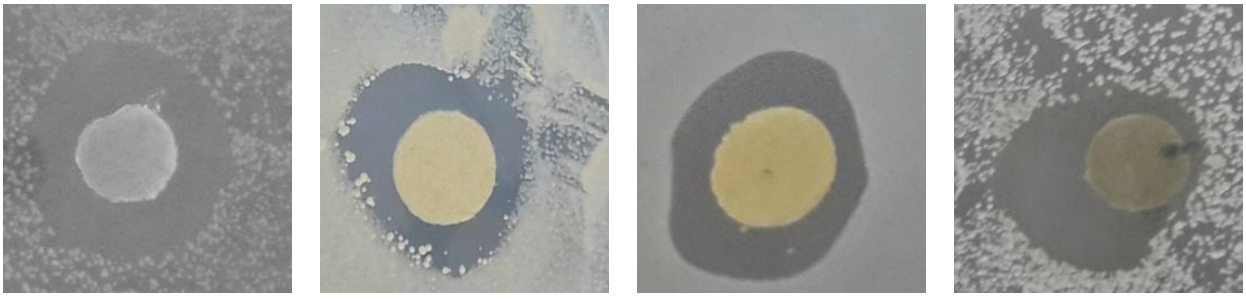
One-way Anova with LSD at 5%; Means that do not share the same letters are significantly different, n= 5
Fig. 2: Quantification of the TPC in the different crude solvent plant extracts



One-way anova with LSD at 5%-; Means that do not share the same letters are significantly different, n= 5
Fig. 3: Quantification of the TFC in the different crude solvent plant extracts



EA: Ethyl Acetate; ME: Methanol; PE: Petroleum spirit; PC: Positive control (Tetracycline); NC: Negative control;
One-way anova with LSD at 5%- n= 5; Means that do not share a letter are significantly different
Fig. 4: Mean inhibition activity (mm diameter) of locally plant extracts against Candida albicans (ATCC1023)



a **b** **c** **D**

Fig. 5: Zone of inhibition (mm diameter) of the some test microorganisms
Escherichia coli 0145:H28 Acc. No.CP006027.1
Viridibacillus arenosi Strain LMG 22166
Staphylococcus aureus ATCC 29213
Candida albicans ATCC1023

nitrate (w/v) was added. After 5 minutes, 150 µL of 10% AlCl3 (w/v) was added. 2000 µL of sodium hydroxide (1 M) was added after 1 minute followed by 1200 µL of distilled water. The mixtures were vortexed and incubated for 30 minutes and the absorbance was measured at 510 nm. A quercetin standard curve was made (0 -200 µg/mL). Total flavonoid content (TFC) was determined as quercetin (QE) equivalents (µg/g of fresh sample weight) (Formula [1]).

The total phenolic content of plant extract was determined using Folin-Ciocalteu reagent method. To 250 µL of Folin-Ciocalteu's reagent, an aliquot of 10 µL of extract sample was added, followed by 3.5mL of deionised water. After 3 minutes, 1mL of 20% sodium carbonate was added. The mixture was vortexed and incubated at 400C for 40 minutes. It was allowed to cool in a dark cupboard and absorbance of the reaction mixtures was measured at 685nm. A gallic acid standard curve was made (0-300 µg/mL). Total phenolic content (TPC) was determined as gallic acid (GAE) equivalents (µg/g of fresh weight) (Formula [1]). The absorbance for both reaction mixes was measured on Jenway spectrophotometer 7305 -UV-Visible.

$$TPC(TFC) = \frac{R\ x\ DF\ x\ V_1}{W\ x\ V_2} \quad \ldots\ldots\ldots\ldots [1]$$

*Triplicate readings were taken for each sample
R = result obtained from the standard curve;
D.F = dilution factor; V_1 = volume of stock solution, V_2=volume of extract used
W = weight of plant material used.

Antimicrobial assay

Antimicrobial susceptibility test (Disc Diffusion Method): The test microorganisms included for this study were six Gram positive (*Bacillus algicola* Acc.13/5, *Bacillus cereus* ATCC 11778, *Listeria innocua* ATCC 33090, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermis* ATCC 12228, *Viridibacillus arenosi* Strain LMG 22166), six Gram negative (*Escherichia coli* ATCC 25922, *Escherichia coli* 0145:H28 Acc. No.CP006027.1, *Klebsiella oxycota* ATCC 43086, *Proteus vulgaris* Strain NCTC 11938, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC14028) and one fungus (*Candida albicans* ATCC 1023). Regular sub-cultures of the test microorganisms were carried out on sterile Nutrient agar plates. The bacteria were grown in nutrient broth (NB) at 37° C for 24-48 hours. All the inocula were standardised by dilution with sterile NB (0.1mL inoculum to 9.9 mL NB) to an absorbance of 0.4-0.6 at 600nm.

Available dehydrated Muller Hinton Agar (MHA) Media (19g/L (w/v)) was prepared and sterilised in an autoclave at 121° C and 15 lb pressure for 15 minutes and poured into to Petri dishes for the disc diffusion test. Circular paper discs of (6 mm diameter, 0.09 mm) were also sterilised. The sterile discs were aseptically placed over plates of MHA (5 discs /plate) already seeded/spread with each of test pathogens (100µL/plate) and were impregnated with equal volume 5 µL of each crude plant extracts at a fixed concentration of 0.2g/mL(w/v). The plates were incubated in an upright position at 37 °C for 24-48 hours and zone of inhibition was measured (in mm diameter). The clinical strains were also tested for their sensitivity against both a positive control (standard antibiotic tetracycline, 30µg) and two negative controls (solvents - Ethyl acetate and Methanol). The sterile discs were also tested on a sterile plate of MHA without extracts and inoculum. Inhibition zones (IZ) with diameter greater than 12mm were considered as having low antimicrobial sensitivity, and those lying between 12 and 16 mm moderately active and above 16 mm as highly active[20].

Antibacterial activity by Microdilution - (Minimum Inhibitory Concentration (MIC) Method): The serial microdilution method was used to determine the minimum inhibitory concentration (MIC) antibacterial activity of fractionated plant crude extracts. Each bacterium was incubated in 10 mL of Mueller Hinton (MHB) broth overnight at 37°C. The overnight cultures were standardised by dilution with sterile MHB (0.1mL inoculum in 9.9 mL MHB) to an absorbance of 0.4-0.6 at 600 nm. 100 µL of the tested sample for each bacterium was two-fold serially diluted with 100 µL sterile distilled water in a sterile 96-well microplate. A two-fold of tetracycline (30µg/mL) was also used as positive control against each bacterium. Methanol, and ethyl acetate were used as negative control and 100 µL of bacterial suspension was added to each well.

The plates were covered and incubated at 37°C for 24 h, and the lowest concentration of the extract causing complete inhibition of the bacterial growth was taken as MIC. The results were compared with that of control using

Table 2: Summary of the antimicrobial sensitivity of the different crude leaf extracts against bacteria

Antimicrobial sensitivity	Microganisms and respective extract
Moderate[12< IZ < 16]	*Bacillus algicola- J. curcas*-EA, *J. integerrima*-EA, *R. communis*-ME
	Escherichia coli 0145 :H28 -*J. curcas*-ME,*J. podagrica*-ME, *R. communis*-EA, *R. communis*-ME
	Escherichia coli- J. podagrica-EA, *R. communis*-EA, *R. communis*-ME
	Listeria innocua - R. communis-ME
	Proteus mirabilis- R. communis-EA
	Salmonella typhimurium- J. curcas-EA
	Staphylococcus aureus- J. curcas-EA, *J. integerrima*-EA
	Staphylococcus epidermis- J. podagrica-ME
High[>16mm]	*Bacillus algicola-- J. multifida*-EA
	Staphylococcus epidermis- J. multifida-EA

IZ: zone of inhibition

Table 3: Mean zone of inhibition of solvent crude extracts from selected medicinal local plants against gram positive and negative microorganisms

Crude Extracts: 0.2mg/mL Plant / Solvents Microorganisms	Mean Zone of inhibition (Mean ± standard deviation in mm diameter)												
	J. curcas L.		*J. integerrima* Jack.		*J. podagrica* Hook.		*J. multifida* L.		*Ricinus communis* L.		NC		PC
	EA	ME	EA	ME	EA	ME	EA	ME	EA	ME	EA	ME	
Gram +ve													
1.*Bacillus algicola* Acc. No. 13/5	15.20± 0.84a *a*	10.20 ± 0.45a *a*	12.60 ± 1.82b *b*	10.20± 0.45 a *a*	10.60± 1.52 a *a*	6.00 ± 0.00 d *d*	16.00± 1.00 b *b*	9.20 ± 0.84 b *b*	10.40± 0.55 b *b*	12.80 ± 1.30a *a*	12.20± 0.84	13.20± 0.84	17.20± 1.48
2. *Bacillus cereus* ATCC 11778	9.00± 0.00 d	6.00 ± 0.00 d	12.00 ± 0.00 c	8.00 ± 0.00	10.00± 0.00 b	9.00 ± 0.00 c	6.00 ± 0.00 d	6.00 ±0.00 c	9.00 ± 0.00 c	8.00 ± 0.00 b	6.00 ± 0.00	6.00 ± 0.00	20.00± 0.00
3.*Listeria innocua* ATCC 33090	10.40± 0.55 b	8.60 ± 0.55 b	11.00 ± 0.71 c	10.60± 0.55 a	8.60 ± 2.07 c	10.80± 0.85 b	12.00± 3.81 c	10.80± 1.10 a	11.20± 0.84 a	14.00 ± 1.73 a	10.00± 0.00	10.00± 0.00	19.00± 0.00
4. *Staphylococcus aureus* ATCC 29213	14.20± 0.84 a	9.60 ± 0.55 a	15.60 ± 1.52 a	8.40 ± 0.89 b	6.00 ±0.00 e	6.00 ±0.00 e	11.40± 1.14 c	9.40 ± 1.34 b	6.00 ± 0.00 d	6.00 ± 0.00 e	12.00± 1.00	13.00± 0.71	19.40± 3.78
5.*Staphylococcus epidermis* ATCC 12228	9.20 ± 0.84 c	6.40 ± 0.89 c	6.00± 0.00 d	6.00 ± 0.00 c	6.20 ± 0.84 d	15.40± 2.41 a	17.20± 1.79 a	10.00± 1.23 a	9.00 ± 0.00 c	8.40 ± 0.89 c	11.00± 1.00	10.60± 1.14	15.40± 2.41
6.*Viridibacillus arenosi* Strain LMG 22166	10.60± 0.89 *b*	10.40 ± 1.34 *a*	11.60 ± 0.89 *c*	9.80 ± 0.84 *b*	11.80± 0.45 *a*	11.20± 0.45 *b*	10.40± 0.55 *c*	8.80 ± 0.45 *b*	9.00 ± 0.71 *c*	11.40 ± 0.55 *b*	10.00± 0.00	9.00 ± 0.00	17.20± 0.45
Gram –ve													
1.*Escherichia coli* ATCC 25922	11.40± 0.89 *a*	11.00 ± 1.41 *b*	9.60± 0.55 *b*	6.80 ± 0.45 *c*	12.20± 1.48 *a*	12.60± 0.55 *a*	10.60± 0.89 *b*	9.80 ± 0.84 *b*	13.00± 1.41 *a*	12.40 ± 2.07 *a*	13.00± 1.23	12.00± 0.71	15.20± 2.39
2.*Escherichia coli* 0145:H28 Acc. No.CP006027.1	11.00± 1.00 *b*	14.80 ± 0.45 *a*	9.20± 0.84 *b*	6.00 ± 0.00 *d*	11.00± 1.41 *a*	12.60± 0.55 *a*	6.00 ±0.00 *c*	11.20± 0.84 *a*	14.00± 1.23 *a*	14.00 ± 0.71 *a*	15.00± 0.00	13.20± 1.10	25.00± 0.00
3.*Klebsiella oxytoca* ATCC 43086	11.00± 0.00 *b*	9.00 ± 0.00 *c*	11.00 ± 0.00 *a*	8.00 ± 0.00 *b*	10.00± 0.00 *b*	8.00 ± 0.00 *c*	12.00± 0.00 *a*	6.00 ± 0.00 *e*	11.00± 0.00 *b*	10.00 ± 0.00 *c*	6.00 ± 0.00	6.00 ± 0.00	19.00± 0.00
4.*Proteus mirabilis* Strain NCTC 11938	12.00± 0.00 *b*	8.00 ± 0.00 *d*	6.00 ± 0.00 *c*	12.00± 0.00 *a*	11.00± 0.00 *a*	6.00 ±0.00 *d*	12.00± 0.00 *a*	9.00 ± 0.00 *c*	13.00± 0.00 *a*	11.00 ± 0.00 *b*	6.00 ± 0.00	9.00 ± 0.00	3.00 ± 0.00
5.*Pseudomonas aeruginosa* ATCC 27853	10.00± 0.00 *b*	12.00 ± 0.00 *b*	11.00 ± 0.00 *a*	9.00 ± 0.00 *b*	8.00 ± 0.00 *c*	10.00± 0.00 *b*	12.00± 0.00 *a*	8.00 ± 0.00 *d*	6.00 ± 0.00 *c*	11.00 ± 0.00 *b*	6.00 ±0.00	6.00 ±0.00	24.00± 0.00

Table 3: Mean zone of inhibition of solvent crude extracts from selected medicinal local plants against gram positive and negative microorganisms

Crude Extracts: 0.2mg/mL	Mean Zone of inhibition (Mean ± standard deviation in mm diameter)												
Plant / Solvents	*J. curcas* L.		*J. integerrima* Jack.		*J. podagrica*		*J. multifida* L.		*Ricinus communis* L		NC	PC	
Microorganisms	EA	ME	EA	ME	EA	ME	EA	ME	EA	ME	EA	ME	
6 .*Salmonella typhimurium* ATCC 14028	12.20± 2.05 *a*	12.00 ± 0.00 *b*	9.80± 0.84 *b*	8.60 ± 0.55 *b*	8.40 ± 0.55 *c*	8.00 ± 0.00 *c*	6.00 ±0. 00 *c*	9.60 ±0. 55 *b*	6.00 ±0. 00 *c*	6.00 ± 0.00 *d*	9.00 ± 0.00	10.2 0± 0.45	19.0 0± 0.00

EA: Ethyl Acetate; ME: Methanol; PE: Petroleum spirit; PC: Positive control (Tetracycline); NC: Negative control; One-way ANOVA with LSD at 5%- n= 5; Means that do not share a letter are significantly different(column)

Table 4: MIC of the solvent crude extracts from selected medicinal local plants against gram positive and negative microorganisms

	MIC(µg) GRAM(+ve)						GRAM(-ve)						
Plants /solvents	1	2	3	4	5	6	7	8	9	10	11	12	13
J.curcas L.:													
ME	5.42	75	100	19.7	100	14.2	3.6	25	100	14.5	37.5	14.5	10.8
EA													8
	2.7	75	50	2.7	37.5	25	10.8 8	3.6	14.5	37.5	75	10.8 8	100
J. integerrima Jack.:													
ME	5.42	50	75	100	100	19.7 5	100	100	10.8 8	75	19.7 5	100	50
EA													
	1.8	25	19.7 5	14.5	100	37.5	25	19.7 5	100	25	37.5	19.7 5	50
J. multifida L.:													
ME	100	100	10.8 8	100	14.5	50	2.7	3.6	100	37.5	100	100	25
EA													
	25	50	7.25	100	25	75	7.25	37.5	37.5	75	19.7 5	14.5	14.5
J.podagrica (Hook). :													
ME	50	100	75	10.8 8	10.8 8	25	25	10.8 8	75	100	100	25	7.25
EA													
	3.6	100	9.05	100	14.5	37.5	3.6	100	14.5	14.5	14.5	37.5	10.8 8
Ricinus communis L.:													
ME	1.8	75	19.7 5	50	100	100	2.7	7.25	9.05	7.25	4.52	100	7.25
EA													
	25	75	3.6	7.25	100	75	3.6	25	3.6	100	8.15	100	10.8 8
Tetracycline(30µg)	0.9	3.6	30	11.2 5	22.5	15	1.8	1.8	30	3.7	1.8	11.1 3	100

1. Bacillus algicola Acc. No. 13/5; 2. Bacillus cereus ATCC 11778; 3. Listeria innocua ATCC 33090; 4. Staphylococcus aureus ATCC 29213; 5. Staphylococcus epidermis ATCC 12228; 6. Viridibacillus arenosi Strain LMG 22166; 7. Escherichia coli ATCC 25922; 8. Escherichia coli 0145:H28 Acc. No.CP006027.1; 9. Klebsiella oxytoca ATCC 43086; 10. Proteus mirabilis Strain NCTC 11938; 11. Pseudomonas aeruginosa ATCC 27853; 12 .Salmonella typhimurium ATCC 14028; 13. Candida albicans (ATCC1023)
ME: Methanol; EA: Ethyl acetate

petroleum, ether methanol, ethyl acetate and tetracycline (antibiotic). The experiment was performed in triplicate. Bacterial growth was assayed with the addition of 40 µL of 0.2mg/mL iodonitrotetrazolium violet (INT) to each well after incubation at 37°C for 30 minutes. Bacterial growth in the wells was indicated by the development of a red colour and colourless wells indicated inhibition by tested extracts.

Toxicity assay: Three-day old larvae of *Bactrocera zonata* and *Bactrocera cucurbitae* were collected from the Entomology Division (Ministry of Agro Industry and Food Security, Réduit). The methanolic and the ethyl acetate crude extracts of *J.curcas, J.integerrima, J.multifida, J podagrica* and *R. communis* were tested for their larvicidal effect on two types of flies larvae at three different doses - 0.2 , 0.4 and 0.8 mg/L (w/v)/extracts. The natural food diet was formulated using 6%(w/v) of maize powder, sugar cane bagasse, waste brewer's yeast and wheat bran respectively , 11% (w/v) sugar, 0.1% (w/v) sodium benzoate, 0.1% (w/v) Nipagen, 0.008%(w/v) hydrochloric acid and 64.8% water. A set of insect larvae was allowed to grow and recovered in the fixed weight of food diet for two days eliminating the risk of natural death in Petri-dishes (100 x 15 mm). Then, the food diet was sprayed with 10 ml of the different concentration of the plant extracts. The experiment was set in a completely randomised manner with 3 replicates per treatments (extracts) and controls (with and without the solvent). The % mortality was recorded at 24, 48 and 72 hours.

Statistical analysis: For the phytochemical and antimicrobial assays, all data were expressed as mean ± standard deviation with one-way ANOVA at 5% and LSD test compared the differences between the means. The mortality assay data collected were subjected to log10 transformation prior to analysis and Probit analysis was used for calculating the lethal doses (LD). Minitab® 16.2.4 and Microsoft Excel 2010 were used for the statistical analyses, and tables and graphs output.

RESULTS

Phytochemical screening

Qualitative tests: Phytochemical and fractions (TLC) screening initially revealed the presence of alkaloids, coumarins, flavonoids, steroids tannins, and phenols in most of the crude extracts. Flavonoids and phenol appeared in both ethyl acetate and methanolic crude extracts. Coumarins were observed only for *J. curcas, J. podagrica* and *Ricinus communis*. Variation in the R_f values (0.42-0.87) obtained indicated the presence of different phenol and flavonoid compounds and for methanolic extract of J. *multifida* (R_f value 0.42) confirmed the presence of flavonoid-glycoside compound (Table 1.0).

Quantification of TPC and TFC: The TPC and TFC of the two solvent crude extracts showed significant differences ($p<0.05$) for the different medicinal plants (Figures 2.0 & 3.0). The quantitative analysis of the phytochemicals of various crude extracts revealed that the total phenol content was higher compared to the flavonoid content and that the ethyl acetate crudes extracts of mature leaves gave better yield of phenol and flavonoid. The ethyl extract of *Ricinus communis* leaves contained the highest amount of TPC (632.33±19.2µgGAE/g FW) followed by *J. integerrima* (408.00±34.00µgGAE/g). Phenol was obtained for both solvents extracts of *J. multifida* only. The TFC for the ethyl extract of leaves of *J. integerrima, J. podagrica* and *Ricinus communis* were 10.14±0.59µgQE/g 7.80±0.89µgQE/g and 7.61±1.88µgQE/g respectively where as the methanolic extracts of *J. integerrima* and *J. multifida* had values of 11.31±0.89µgQE/g and 7.70±0.19µgQE/g.

Antimicrobial Assay

Antibacterial and antifungal susceptibility test: Most of the 10 crude extracts of the five plant species had significant varying degrees of antibacterial and antifungal potential ($P<0.05$) (Table 2.0 & Figure 3.0). The antibacterial activity of all extracts depends largely upon the type of solvent used for extraction and the bacterial strains tested in the susceptibility assay. The ethyl acetate extracts exhibited promising antibacterial activities against gram positive test microorganisms compared to the methanolic extracts. The extract from leaves of *J. multifida* showed highest antibacterial activity with a mean zone of inhibition of 17.20±1.79 mm diameter against *Staphylococcus epidermis* (ATCC 12228) and 16.00±1.00mm diameter for *Bacillus algicola* Acc. No 1/3

Table 5: Lethal dose (LD) of the different crude solvent extracts against Diptera species

	Bactrocera cucurbitae		*Bactrocera zonata*	
	LD_{50}	LD_{90}	LD_{50}	LD_{90}
Plants / solvents	g/L			
J. curcas L.: ME	0.18	0.97	2.75	6.69
EA	0.65	3.32	--	--
J. integerrima Jack.: ME	0.38	1.93	0.24	0.94
EA	2.25	11.48	0.82	3.23
J. multifida L.: ME	0.36	1.86	0.17	0.66
EA	0.35	1.00	<0.01	<0.01
J. podagrica (Hook). : ME	0.29	1.46	0.39	1.54
EA	2.44	12.30	0.41	1.62
Ricinus communis L.: ME	0.22	1.12	2.88	11.29
EA	1.93	9.77	--	--

No. of larvae/treatment/replicate (n) = 10 (n=3) (Probit analysis): -- no death observed:
LD_{50} & D_{90}: Dose level at which 50% and 90% larvae death

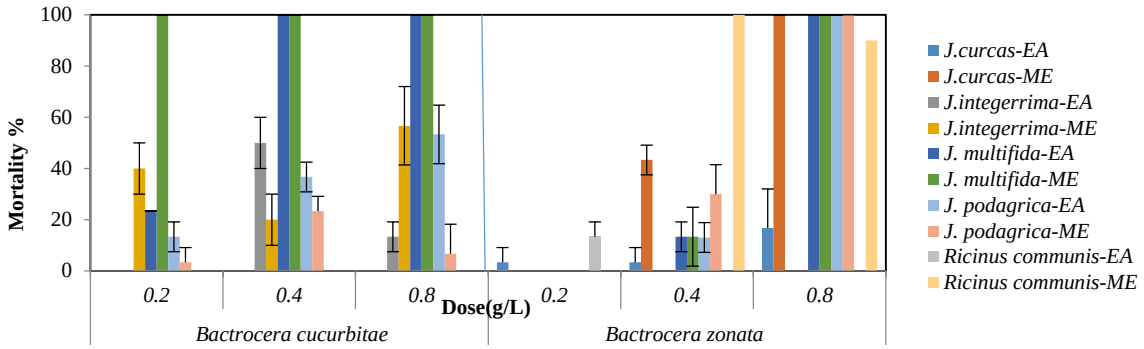


Fig. 6: Mortality% of both larvae for the different of concentration of crude solvent extracts after 24 hours Mean ± standard deviation with LSD at 5%, n=10, controls: For all treatments with and without solvents, death of the larvae were not observed

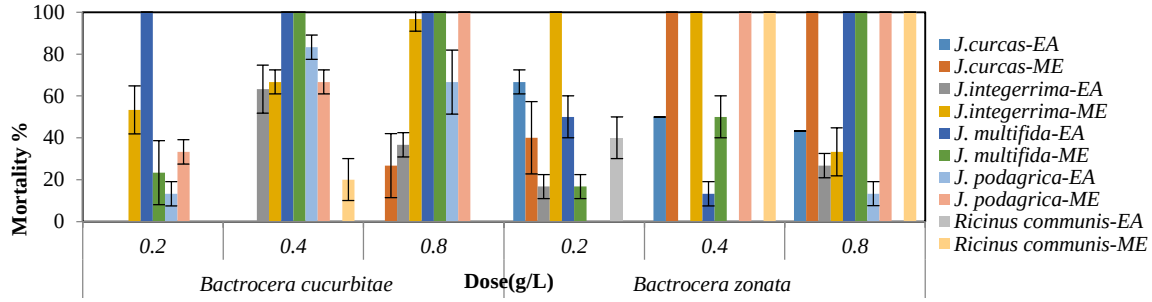


Fig.7: Mortality% of both larvae for the different of concentration of crude solvent extracts after 48 hours Mean±standard deviation with LSD at 5% n=10 controls: For treatments with and without solvents, death of the larvae were not observed

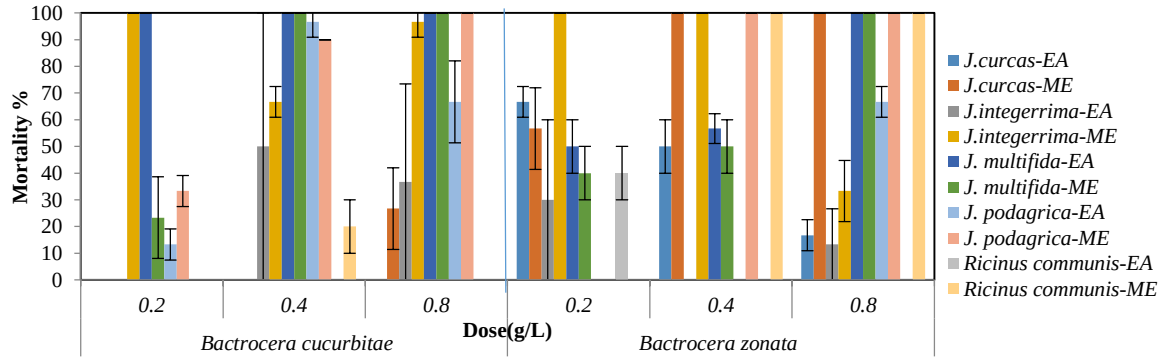


Fig.8: Mortality% of both larvae for the different of concentration of crude solvent extracts after 72 hours Mean±standard deviation with LSD at 5% n=10 controls: For treatments with and without solvents, death of the larvae were not observed

followed by *J. integerrima* and *J. curcas* with a mean zone of inhibition of 1560±1.52 mm for *Staphylococcus aureus* (ATCC 29213) and 15.20±0.84 mm for *Bacillus algicola* Acc. No. 13/5 respectively. Moreover, methanolic extract of *J. curcas* leaves had a mean inhibition zone of 14.80±0.84 mm for gram negative

Escherichia coli 0145:H28 Acc. No.CP006027.1 (Figure 5.0and Table 4.0), *Proteus mirabilis* Strain NCTC 11938 and *Escherichia coli* ATCC 25922 were both equally susceptible to methanol extract of *Ricinus communis*.

Highest means for the antifungal effect of *Candida albicans* (Figure 4.0 and 5.0) were obtained for both extracts of *J. multifida*, 14.40±0.1 and 11.8±0.45 mm diameter, followed by the ethyl acetate extracts of *Ricinus communis* (10.40±1.34 mm diameter) and *J. curcas* (9.6±0.55 mm diameter). The susceptibility test also showed that the test microorganisms were more sensitive to the crude plant extracts than the standard antibiotic, tetracycline.

Antimicrobial activity (MIC): The antimicrobial activity of the ethyl acetate crude extracts seemed to be more effective than the methanolic crude extracts for inhibiting microorganism activity for both Gram(+ve) and Gram(-ve) strains (Table 3.0). The most significant antimicrobial activity which were lower than the standard antibiotic Tetracycline, were noted for methanolic extracts of *J. curcas* (2.7 µg/L) against *Staphylococcus aureus* ATCC 29213 and minimum concentration of 3.6µg/L for *Ricinus communis* ethyl acetate crude extracts against *Listeria innocua* ATCC 33090 and *Klebsiella oxytoca* ATCC 43086. Most of the crude solvent extracts had an antifungal potential for *Candida albicans* (ATCC1023 (Table 3.0). The crude solvent extracts had a significant effect on the antimicrobial activity of the following strains- *Listeria*

innocua ATCC 33090, *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermis* ATCC 12228, *Viridibacillus arenosi* Strain LMG 22166, *Klebsiella oxytoca* ATCC 43086, *Salmonella typhimurium* ATCC 14028, *Candida albicans* (ATCC1023) compared to the positive control, Tetracycline.

Crude solvent extracts: toxicity bioassay against *Bactrocera spp*.: The effect of the different concentrations (LD) of the crude extracts varied significantly on the two Diptera larvae. The crude ethyl acetate extract of *J. multifida* L. demonstrated greater efficiency for larval control of *Bactrocera zonata* with LD 90 of less than 0.0l g/L(Table 5.0). *Bactrocera cucurbitae* larvae were more susceptible to the methanolic extracts of *J.curcas* L. (LD50 = 0.18g/L and LD90 = 0.97g/L) and *Ricinus communis* L (LD50 = 0.22g/L and LD90 = 1.12g/L)(Table 6.0). The mortality % increased with time for the different concentration of the crude extracts (Figure 7.0-9.0). 3.3% t0 100% larvae were killed after 24 hours. The insecticidal effect on *Bactrocera zonata* was 100% after 24 hours at the dose of 0.8 g/L of crude solvent extracts of *J. curcas, J. multifida, J. podagrica* and *R. communis.* 100% mortality was also noted for *Bactrocera cucurbitae* for crude methanolic extract of *J. multifida* at a dose of 0.8g/L. At 48 and 72 hours most of the Diptera larvae died. The mortality % ranged from 13.3 to 100 % for both Diptera larvae after 48 and 72 hours (Figures 6.0-8.0).

DISCUSSION

Both *Ricinus communis* and *Jatropha* species are used in local traditional medicine for example castor oil is used as purgative. Crude extracts of the leaves from the different species of *Jatropha* present on the island of Mauritius were never studies previously for their presence of phytochemical compounds, antimicrobial/antifungal properties and their ability to act as natural insecticides.

The phytochemical screenings were very dependent on the type of solvent used. This observation is in line with the findings ofSrinivasan et al.[21] and Kordali & Cakir[22] who reported that different solvents have different spectrum of solubility for the phytoconstituents. In *J. curcas* and *Ricinus communis* coumarins were present only in the ethyl acetate extract and in the methanolic extract of *J. podagrica*. Flavanoids on the other hand were present in both ethyl acetate extract and methanol. RF (retention factor) at different distance on the TLC plates and the computed R_f values indicated the presence of different flavonoids and phenolic compounds in the leaves crude extracts. Although some phytochemical studies conducted by Jitendra and Kumar[23] reported the presence of saponins in *Ricinus communis*, and in *Jatropha curcas* by El Diwani et al.[24], in this study saponins were not detected. Although the absence of alkaloids in *Jatropha curcas* leaf extracts had been reported by Kubmarawa et al.[25], it was Igbinosa[9] and Akinpelu et al[26] who observed the presence of alkaloids in *J. curcas* stem bark and leaves extracts respectively, and the present study also confirms the presence of alkaloid in the leaf extracts. Farooq et al.[27] stated that as plants occur in varying habitats, the great magnitude of variation in the concentration and composition of phytochemical ingredients in the different parts of these plants can be explained.

These compounds have long been associated with medicine and were reported as efficient therapeutical substances. They exert considerable antimicrobial activity through different mechanisms[28]. Antimicrobial activity of the *Jatropha* species have been widely reported, but not all the leaf materials have been studied. In *J. multifida* although the root does not inhibit the growth of *Candida albicans*, this study found that the leaves of *J. multifida* do inhibit the growth of the fungus at an MIC of 25 µg/L when using methanol solvent and 14.5 µg/L for ethyl acetate solvent[29]. The crude methanolic extract of the leaves of *J. curcas* inhibited the growth of *Staphylococcus aureus, S. epidermidis, Pseudomonas aeruginosa, Escherichia coli, Bacillus cereus, B. subtilis* and *Proteus vulgaris* as observed by Igbinosa[9] for the crude root extracts. Therefore it can be said that the J.curcas leaves has the same antimicrobial properties as the stem bark.

Several phytochemicals, in particular the flavones have the potential to interact with the vertebrate oestrogen receptor[30]. The flavones, apigenins, orientin, vitexin, vicenin II and biflavone were isolated from the leaves of *J. curcas*. In this study these flavones have also proved to be toxic against the Diptera species used, hence inducing a high mortality rate.

CONCLUSION

Interesting results were obtained during this study, showing the effect of solvent on phytochemical extraction, crude solvent extracts affecting bacterial growth and finally the insecticidal properties of crude leaves extracts were tested on two economically important plant pests in Mauritius, thereby paving way for more research on *J. multifida and Ricinus communis*.

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