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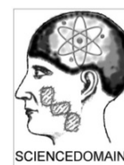

Antifungal Activity and Phytochemical Screening of Different Solvent Extracts of *Euphorbia tirucalli* Linn.

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Authors' contributions

This work was carried out in collaboration between all authors. Author SS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors CCK and EA managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The antifungal effect of *Euphorbia tirucalli* L. stem and root extracts was evaluated on microbial strains of *C. albicans* (ATCC 9002) and *A. niger.* Plant parts were serially extracted by using petroleum ether, chloroform, methanol and aqueous in the increasing order of their polarity. The minimum inhibitory concentration (MIC) and phytochemical screening for the different plant parts and solvents used was also performed. The antimicrobial activity of the plant extract was assayed using the agar plate disc diffusion. The extracts inhibited the growth test organisms at different concentrations with significant higher antifungal activity revealed in high concentration than lower one. The significant antifungal activity was achieved in methanol and aqueous extracts which had mean inhibition zone of 15.33±0.88 mm and 17.33±0.33 mm respectively for *C. albicans* (ATCC 9002) while *A. niger* had 14.67±0.67 and 16.33±0.33 for methanol and aqueous respectively. The methanol and aqueous had better scores for antimicrobial activity compared to other solvents used. The minimum inhibitory concentration ranged from 1.97 mg/ml to 2.52 mg/ml for methanol and 2.35

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mg/ml to 3.44 mg/ml for aqueous extracts. Phytochemical screening revealed the presence of alkaloids, tannins, saponins and flavonoids in the extracts. The antifungal activity of the crude stem and root extracts of revealed in this study is an indication of antifungal potential of *E. tirucalli* which may be employed in the management of microbial infections forms a basis for more investigation on phytochemical compounds to be useful for drug development.

Keywords: Euphorbia tirucalli; microbial strains; antifungal activity; solvent extracts; phytochemical screening.

1. INTRODUCTION

Euphorbia tirucalli L. is a shrub or a small tree that can grow up to 7 – 12 m high which belongs to the genus *Euphorbia*, one of the 8,000 species within family *Euphorbiaceae* widely distributed in semi-arid areas of tropical and most sub-tropical areas [1]. The species is characterized with pencil-like branches which have inspired its English name, the pencil-tree whereas its vernacular name is known as Masongorwa (Jita, Sukuma) and Swahili names known as Minyaa Mtovua macho or Mwasi [2,3]. The plant bears white poisonous latex, which accounts for its low herbivore pressure and medicinal features [4,5]. *Euphorbia tirucalli* is one of the most important trees usually planted for boundary demarcation but also as a live fence around compounds, shrines and kraals due to its ability to withstand extreme aridity stress [2].

Expenditure on medicines accounts for a major proportion of health costs in developing countries. It is estimated that one-third of the developing world's people are unable to receive or purchase essential medicines on a regular basis. This means that access to treatment is greatly dependent on the availability of affordable medicines. The World health Organization (WHO) estimates that about 80% of the population living in the developing countries depends almost entirely on traditional medicine for their primary healthcare needs [6]. Fungal infections contribute comprehensively to human morbidity and mortality and its impact on human health is not largely appreciated. Moreover, concerted research for efficient and effective new drugs and vaccines of human fungal infections lags behind diseases caused by other pathogens [7].

The search for new antifungal compounds is particularly important in order to reduce a significant cause of morbidity and mortality worldwide. In addition, the fungal opportunistic infections such *Candida* and *Aspergillus* species are mainly prevalent in immune compromised patients causing many deceases [8]. Fungal diseases such as *Candida* strains with multiple antibiotic resistances is increasing worldwide, therefore search for alternative remedies are urgently needed as an effective means of treatments for infection of these pathogens [9]. The development of drug resistant pathogens requires new strategies which incorporates indigenous plants as reservoirs of novel antifungal. In an effort to discover new lead compounds many researchers have screened plant extracts to detect secondary metabolites with appropriate biological activities to be used as drugs in their original or semisynthetic form [10-12].

Ethnomedicinal information reported on *E. tirucalli* has shown the potential of treating various ailments such as swelling, asthma, cough, skin problems and rheumatism [13]. Other reported curative ability is its latex is being latex being used against sexual impotence, warts, toothache, hemorrhoids, snake bites and cough among others [3,14]. Furthermore in efforts to explore medicinal potential of *E. tirucalli*, it has been reported its latex and other plant parts have pesticidal effect against pests as such aphids (*Brevicoryne brassicae)* and mosquitoes *Aedes aegypti* and *Culex quinquefasciatus* [15-17]. The species also has been reported to exhibit antioxidant potential, antitumor and antimicrobial activity [18-20].

Although some studies have reported on antibacterial and antifungal activity *E. tirucalli* using different solvents for the extraction [18,19,21,22], little has been done in evaluation of antimicrobial activity by the use of different solvents with serial extraction in the increasing order of their polarity. The serial extraction in order of polarity is very important for obtaining phytochemical components with high antimicrobial activity which might not be extracted serially with solvent of lower polarity. The aim of this study was to analyze the effect of the serial extraction for different solvents of increasing varying polarity (petroleum ether,

chloroform, methanol and aqueous) on antifungal activity and phytochemical contents to provide a reference for the comprehensive development and utilization of the medicinal potential of *E. tirucalli.*

2. MATERIALS AND METHODS

2.1 Sample Collection

The fresh plant of *Euphorbia tirucalli* was collected from Kimara, Mbezi, Dar es Salaam, Tanzania at the area located along Latitude 6° 45' 57'' South and Longitude 39° 9' 37'' East, at Altitude of 68.0 meters. The identity of a plant was confirmed by botanist and the voucher specimen deposited in the herbarium at the Department of Botany, University of Dar es Salaam.

2.2 Root and Stem Crude Extracts

The root and stem were separately washed with running tape water four times and were cut into small chips. Then these small pieces were rinsed with distilled water and air dried for five days and then was dried in hot air oven at 50℃ for three days. The dried pieces of root and stem were separately ground into fine uniform powder using a Thomas- Willey milling machine. Then weight of 40 gm of root and stem were separately mixed with 100 ml of solvent and serially extracted by using petroleum ether, chloroform, methanol and aqueous in the increasing order of their polarity for 48 hours. The extraction was carried out by cold maceration process. The extracts were filtered using Whatman filter paper No. 1 (125 mm). The petroleum ether, chloroform, methanol and aqueous filtrates were separately collected in the three different beakers and concentrated in room temperature with ceiling fan for 24 hours to obtain crude extracts. The aqueous filtrates were concentrated in rotary evaporator at 50℃ for 3 hours to obtain crude extracts.

2.3 Microorganisms and Inoculum Preparation

Strains of fungi, *Candida albicans* (*ATCC 9002*) and *Aspergillus niger* (local isolates from coffee wastes) were used as for antifungal activity. These strains were obtained from the Department of Molecular Biology and Biotechnology, University Dar es Salaam. All strains were sub-cultured onto Sabouraud Dextrose Agar (SDA) to ensure purity and viability. Inoculum was prepared by picking colonies from a 24 hour old culture and was suspended in 5 ml of sterile 0.145 mol/L saline (8.5 g/L Nacl; 0.85% saline). The resulting suspension was vortexed for 15 sec and its turbidity was adjusted visually with turbidity of 0.5 McFarland standard. This yielded a yeast stock suspension of 1.5 x 10^{-6} cells per mL. McFarland standard was prepared by adding 0.5 mL of 0.048 M $BaCl_2$ (1.17% w/v $BaCl_2$) to 99.5 mL of 0.18 M H_2SO_4 (1% v/v) with constant stirring.

2.4 Agar Well-Diffusion Assay and Evaluation of Antifungal Activity

Agar well diffusion techniques was used to determine and compare antifungal activities of the root and stem extracts from *E. tirucalli* of different solvent extracts. A standardized concentration of inoculum of 100 µl was poured into a conical flask contained 300 ml of molten SDA at around 40℃ and mixed well. The inoculated agar was then poured into 10 plates and allowed to solidify. The holes with 6mm in diameter were punched with a sterile cork borer aseptically in the agar plate. The sample extract for antifungal assay was prepared as explained by Usman et al. [21], with some modifications. Weight of each pure crude extract obtained was separately dissolved in vials each contained 5ml of 10% DMSO to make stock solution of 100% concentration of each crude extract. Different concentrations of 60%, 80% and 100% were made by dilution formula; the experiment was performed in triplicate. 50 µL of each of this dilution was poured into the ditch hole bored onto the agar. The Dimethyl Sulfoxide *(*DMSO*)* was used as negative control and Clotrimazole was used as positive control. The inoculated agar plates were then incubated at 31℃ for 24 hours. Evaluation of antifungal activity was done through measuring diameter of the zone of inhibition and the average diameter in millimeter for each sample was calculated.

2.5 Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) was determined through preparation of concentrations of 10%, 15%, 20%, 40%, 60%, 80% and 100%. The crude extracts were made by pipetting the volume in µL of crude extract stock solution and adding to a glass vial containing the amount of DMSO. For example 20% concentration was made by pipetting 200 µL of crude extract stock solution and adding to a glass vial contained 800 µL of 10% DMSO and 40% concentration was made by pipetting 400 µL of crude extract stock solution and adding to

a glass vial contained 600 µL of 10% DMSO. The fungal strains were cultured in Sabouraud Dextrose Agar plates and 50 µl of each of these concentrations were put into well punctured on agar plates. The agar plates were then incubated at 31℃ for 24 hrs. 50 µL of Clotrimazole was used as a control and this 50 µL was put into one well in each agar plate. After incubation the zones of inhibition were examined; the concentration which showed the smallest zones of inhibition was regarded as the MIC value.

2.6 Phytochemical Analysis

The qualitative phytochemical analysis was done as explained for standard procedures for qualitative tests for the solvent extracts [23,24]. It was screened for alkaloids, steroids, flavonoids, saponins, tannins, phenolic compounds, diterpenes and cardiac glycosides.

2.7 Statistical Analysis

All the experiments were conducted in triplicate and statistical analysis of the data was performed by analysis of variance (ANOVA) using SPSS 20 software. All data were presented as mean values and standard mean errors for the replicate sets of experiments in each case.

3. RESULTS

3.1 Determination of Inhibition Zone of Different Solvent Extracts of Different Concentrations

In this study wells on the agar plate were used as point source and crude extracts were used as an antimicrobial agent. The inhibition zones of different solvent extracts are indicated in the tables 1, 2, 3 and 4 for petroleum ether, chloroform, methanol and aqueous respectively. The results for petroleum ether in different concentration tested indicated that no inhibition was achieved as antifungal except for the clotrimazole as positive control for both *C. albicans* (ATCC 9002) and *A. niger* (Table 1). The DMSO (Dimethyl sulfoxide) which was used as negative control for both *C. albicans* (ATCC 9002) and *A. niger* also didn't indicate any inhibition zone. For chloroform extract it was only for the root part revealed that antifungal activity with inhibition zone of 11.33±0.88 for the crude concentration at 100% for *A. niger* (Table 2).

Methanol extracts revealed antifungal activity for both root and stem parts with high inhibition zone of towards high concentrations. At concentration from 60% to 100% the inhibition zone for stem extract ranged from 11.33±1.20 to 14.33±0.66 for *C. albicans* (ATCC 9002) while for root extracts it ranged from 12.66±0.33 to 15.33±0.88 (Table 3). The antifungal activity of methanol extracts for *A. niger* was slightly low compared to *C. albicans* (ATCC 9002) at higher 100% and ranged from 13.67±0.88 to 14.67±0.67 for stem and root extracts (Table 3). The aqueous extracts had the average high antifungal activity for both root and stem parts with high inhibition zone of towards high concentrations for both *C. albicans* (ATCC 9002) and *A. niger* (Table 4). At higher concentration 100% for stem extract the inhibition zone for *C. albicans* (ATCC 9002) was 14.33±0.66 while for root extract it was 17.33±0.33. Also for *A. niger* the high inhibition zone was 16.33±0.33 for stem extract while for root extract it was 14.67±0.67 (Table 4).

Table 1. Mean zone of inhibition (in mm) of petroleum ether stem and root extracts of *E. tirucalli*

Petroleum ether			
Extract type	**Crude Conc (%)**	**Test organism**	
		***C. albicans* (ATCC 9002)**	***A. niger* (local isolates)**
Stem	60	0.00±0.00	0.00±0.00
	80	0.00±0.00	0.00±0.00
	100	0.00±0.00	0.00±0.00
	Control	24.80±0.37	33.60±0.51
Root	60	0.00±0.00	0.00±0.00
	80	0.00±0.00	0.00±0.00
	100	0.00±0.00	0.00±0.00
	Control	24.80±0.37	33.60±0.51

Table 2. Mean zone of inhibition (in mm) of chloroform stem and root extracts of *E. tirucalli*

Chloroform			
Extract type	**Crude Conc (%)**	**Test organism**	
		***C. albicans* (ATCC 9002)**	***A. niger* (local isolates)**
Stem	60	0.00±0.00	0.00±0.00
	80	0.00±0.00	0.00±0.00
	100	0.00±0.00	0.00±0.00
	Control	24.80±0.37	33.60±0.51
Root	60	0.00±0.00	0.00±0.00
	80	0.00±0.00	9.67±1.20
	100	0.00±0.00	11.33±0.88
	Control	24.80±0.37	33.60±0.51

3.2 Comparison of Antifungal Activity of Different Solvent Extracts

The comparison of antifungal activity of different solvent extracts was done by comparing the average zones of inhibition shown by different solvent extracts as indicated in tables and Fig. 1. In average both methanol and aqueous revealed highest zone of inhibition indicating high antifungal activity for for both *C. albicans (ATCC 9002)* and *A. niger* compered to petroleum ether and chloroform (Fig. 1). Fig. 2 is shows the antifungal activity for the different plant parts tested, the results indicates that extracts from the stem had the same effect for both *C. albicans (ATCC 9002)* and *A. niger* while root extract had higher antifungal activity for *A. niger* than *C. albicans (ATCC 9002).*

3.3 Determination of Minimum Inhibitory Concentration (MIC)

The result for minimum is shown in Table 5. The methanol and aqueous had better scores compared for antimicrobial activity compared to other solvents which showed the minimum inhibitory concentration in range from 1.97 mg/ml to 2.52 mg/ml for methanol and 2.35 mg/ml to 3.44 mg/ml for aqueous extracts (Table 5).

3.4 Qualitative Phytochemical Analysis of Crude Extracts from *Euphorbia tirucalli*

Evaluation of phytochemicals such as alkaloids, flavonoids, glycosides and saponins revealed the presence of most of the constituent in polar extracts such as ethanol, methanol, and aqueous extracts compared with nonpolar extracts mainly petroleum ether and chloroform (Table 6).

Table 3. Mean zone of inhibition (in mm) of methanol stem and root extracts of *E. tirucalli*

Methanol			
Extract type	**Crude Conc (%)**	**Test organism**	
		***C. albicans* (ATCC 9002)**	***A. niger* (local isolates)**
Stem	60	11.33±1.20	11.67±0.88
	80	12.67±0.88	12.67±0.88
	100	14.33±0.66	13.67±0.88
	Control	24.80±0.37	33.60±0.51
Root	60	12.66±0.33	10.33±0.33
	80	13.67±0.67	11.33±0.88
	100	15.33±0.88	14.67±0.67
	Control	24.80±0.37	33.60±0.51

Table 4. Mean zone of inhibition (in mm) of aqueous stem and root extracts of *E. tirucalli*

Aqueous			
Extract type	**Crude Conc (%)**	**Test organism**	
		***C. albicans* (ATCC 9002)**	***A. niger* (local isolates)**
Stem	60	11.67±0.33	11.33±0.33
	80	13.67±0.67	14.67±0.88
	100	14.67±0.67	16.33±0.33
	Control	24.80±0.37	33.60±0.51
Root	60	10.33±0.88	12.33±0.88
	80	15.33±0.33	14.33±0.67
	100	17.33±0.33	15.67±0.33
	Control	24.80±0.37	33.60±0.51

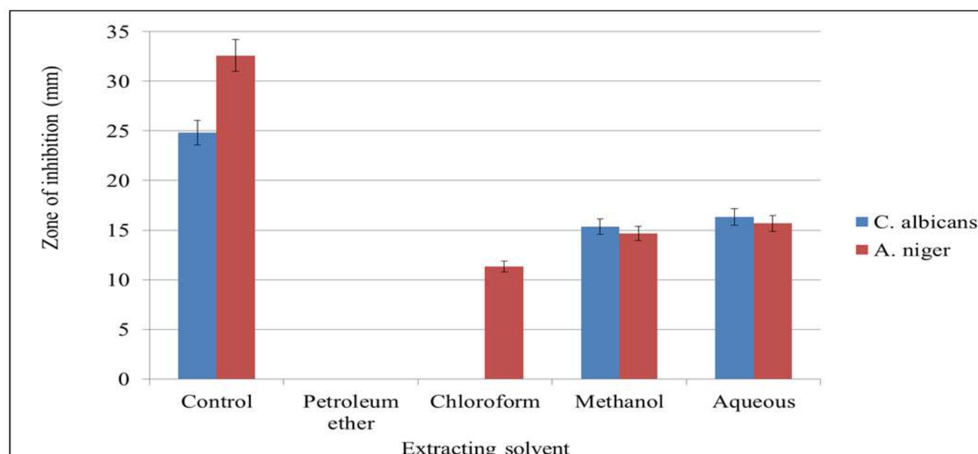

Fig. 1. The average zone of inhibition (in mm) of for different solvent extracts revealing antifungal activity of *E. tirucalli*

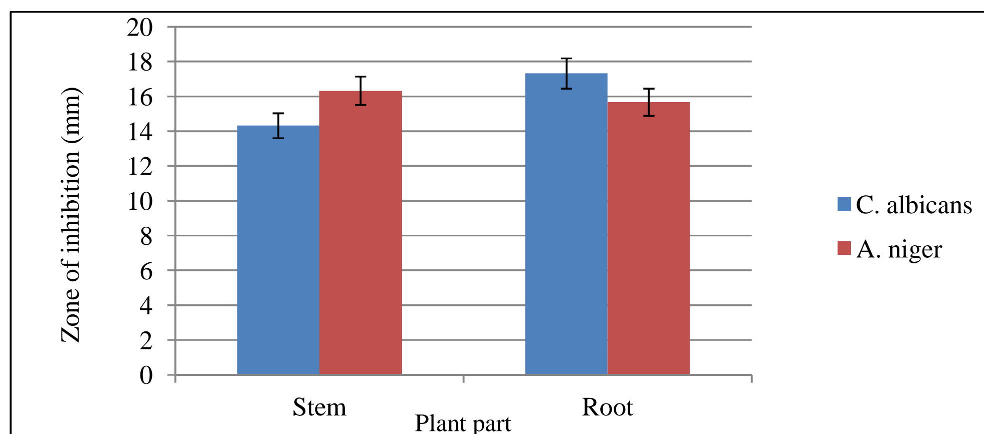

Fig. 2. The average zone of inhibition (in mm) for plant parts tested revealing antifungal activity of *E. tirucalli*

Table 5. Minimum inhibitory concentration (MIC) for the different solvents

Test Organism	Plant part	Type of solvent extract and MIC			
		Petroleum ether (mg/ml)	Chloroform (mg/ml)	Methanol (mg/ml)	Aqueous (mg/ml)
C. albicans	Stem	-	-	1.97	2.35
(ATCC 9002)	Root	-	-	2.52	3.59
A. Niger	Stem	-	-	1.97	2.35
	Root	0.18	0.11	2.52	3.44

Table 6. Phytochemical analysis of *E. tirucalli* crude extracts through different solvents

Part	Alkaloids	Steroids	Flavonoids	Saponins	Tannins	Phenol	Diterpenes	Cardial glycosides
Petroleum ether extract								
Stem	-	-	-	-	-	-	-	-
Root	+	-	+	-	-	-	-	-
Chloroform extract								
Stem	-	+	-	-	+	-	-	-
Root	+	+	-	-	+	-	-	-
Methanol extract								
Stem	+	+	+	-	+	-	-	+
Root	+	+	+	-	+	-	-	+
Aqueous extract								
Stem	+	+	+	-	-	-	+	-
Root	+	+	+	+	-	-	-	-

Key: Negative (-) = absence, Positive (+) = present

4. DISCUSSION

The antimicrobial activity screened in this study revealed that that methanol and sequential with aqueous extract showed significant antifungal activity against all fungal strains tested. As reveled in this study, there was a significant difference in the antifungal properties for the different extracts of *Euphorbia tirucalli* which could be due to the variation in phytochemical constituents with antifungal activity as previously reported in other studies [25]. Methanol and aqueous solvent extracts revealed high presence of phytochemical constituents for potential antifungal activity compared to other solvent extracts. For the tested concentration, the high concentration had high inhibition zone compared to the low concentration being an indication that at high concentration the antifungal activity is more pronounce than lower concentration. The

highest inhibition zone revealed by clotrimazole used as control (positive control) compared to the plant extracts could be due to its effectiveness as an antifungal medicine. When using clotrimazole for example for the case of candida, it works by inhibiting ergosterol, an essential component of the fungal cell walls causing them to break down and allows essential components of the fungus to be released, essentially killing it [26,27]. The DMSO (Dimethyl sulfoxide) which was used as negative control had no any phytochemical constituents thus had no any indication for antifungal activity.

It is also possible as revealed in this study that some compound may not be soluble in petroleum ether, chloroform, methanol and water. The finding from this study is agreement with previous reported study by Madane et al. [28] in assessing the antifungal activities of *Cassia fistula.*

Previous study on extracts of latex of *E. tirucalli* has also been reported to reveal significant antibacterial activity for various bacteria strains and antifungal such as *Candida albicans* when aqueous extract was used compared to the chloroform [29,30]. Some authors have suggested that the healing this property is explained by the action of phytoconstituents of this plant particularly the presence of tannins, steroids and flavonoids [20,29]. These phytochemical constituents screened namely alkaloids, tannins and steroids were also revealed the in the present study through methanol and aqueous extracts.

The active principles responsible for the therapeutic effects of medicinal plants are phytochemicals, typically secondary metabolites, including but not limited to alkaloids, steroids, flavonoids, terpenoids and tannins [31]. The presence of these phytochemicals in polar extracts such methanol and aqueous extracts being responsible for antifungal activity against has also previously been reported in other species when compared to other solvent extracts [9,29]. The reason for this slight difference may be attributed to the solubility level of the phytoconstituents in the solvents. It means that the methanol and aqueous extracts dissolved more active ingredients compared to other solvents.

The different plant parts tested namely stem and root revealed almost equal antifungal activity and this could be due to the presence of rich phytochemicals in both parts. Though all parts of the plant might have the active compounds, the findings from this study differs from other studies on antifungal activity for the Euphobiaceae family which have reported larger inhibition zones for roots extract than stems [32,33]. Such variation could as well be attributed to the variation in phytochemical composition in the tested plants parts in which one part either leaves, stem or roots having more phytoconstituents than the other parts as reported of others species in Euphobiaceae [34,35].

5. CONCLUSION

This study has demonstrated that the methanol and aqueous extracts of *Euphorbia tirucalli* have antifungal activity on *C. albicans* and *A. niger* which are of human pathogenic interest thus further confirms the ethno pharmacological uses of the plant. On the basis of the findings obtained, it is concluded that the solvent extracts of methanol and aqueous for both stem and root should be applied in inhibiting fungal infections. The study also revealed that various phytochemical constituents like alkaloids, steroids and tannins present in the crude extracts. Thus further works on the types of phytoconstituents is needed to identify bioactive components which can be of interest for pharmacological effects and drug design.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Van Damme PLJ. *Euphorbia tirucalli* for high biomass production. In: A. Schlissel and D. Pasternak (Eds.), Combating desertification with plants. Kluwer Academic Pub. 2001;169-187.
2. Mwine TJ, Van Damme P. *Euphorbia tirucalli* L. (Euphorbiaceae): The miracle tree: current status of available knowledge. Scientific Research and Essays. 2011;6(23): 4905-4914.
3. Maregesi SM, Ngassapa OD, Pieters L, Vlietinck AJ. Ethnopharmacological survey of the Bunda district, Tanzania: Plants used to treat infectious diseases. Journal of Ethnopharmacology. 2007;113(3):457-470.
4. Silva ACP, de Faria DEP, Borges NBD, de Souza IA, Peters VM, Guerra MD.

Toxicological screening of *Euphorbia tirucalli* L. Developmental toxicity studies in rats. Journal of Ethnopharmacology. 2007;110: 154-159.

5. Mwine TJ, Van Damme P, Hastilestari BR, Papenbrock J. *Euphorbia tirucalli* L. (Euphorbiacaea): The miracle tree: Current status of knowledge. African Natural Plant Products, Volume II: Discoveries and Challenges in Chemistry, Health, and Nutrition. 2013;1127:4-17.
6. WHO. Traditional medicine: Growing needs and potential. WHO Policy Perspectives on Medicines. World Health Organization, Geneva; 2002.
7. Brown GD, Denning DW, Gow NA, Levitz SM, Netea MG, White TC. Hidden killers: Human fungal infections. Science Translational Medicine. 2012;4(165):165-165.
8. Richardson MD. Changing patterns and trends in systemic fungal infections. Journal of Antimicrobial Chemotherapy. 2005;56:5-11.
9. Panda SK, Brahma S, Dutta SK. Selective antifungal action of crude extracts of *Cassia fistula* L.: A preliminary study on *Candida* and *Aspergillus* species. Malaysian Journal of Microbiolology. 2010;6:62-68.
10. Pelaez F. The historical delivery of antibiotics from microbial natural products—Can history repeat? Biochemical Pharmacology. 2006;71(7):981-990.
11. Lam KS. New aspects of natural products in drug discovery. Trends in Microbiology. 2007;15(6):279-289.
12. Salim AA, Chin YW, Kinghorn AD. Drug discovery from plants. Bioact Mol Med Plants. 2008;24:380-91.
13. Upadhyay B, Singh KP, Kumar A. Ethnomedicinal, phytochemical and antimicrobial studies of *Euphorbia tirucalli* L. Journal of Phytology. 2010;2(4):65-77.
14. Schmelzer GH, Gurib-Fakim A. Medicinal plants. Plant Resour. Trop. Afr. 2008;412-415.
15. Tiwari S, Singh A. Biochemical stress response in freshwater fish *Channa punctatus* induced by aqueous extracts of *Euphorbia tirucalli* plant. Chemosphere. 2006;64:36-42.
16. Rahuman A, Gopalakrishnan G, Venkatesan P, Geetha K. Larvicidal activity of some Euphorbiaceae plant extracts against *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). Parasitology Research. 2008;102:867-873.
17. Mwine J, Van Damme P. Evaluation of selected pesticidal plant extracts against major cabbage insect pests in the field. Tropentag 2010: "World Food System – A Contribution from Europe", ETH Zurich, Switzerland; 2010.
18. Valadares MC, Carrucha SG, Accorsi W, Qquiroz MLS. *Euphorbia tirucalli* L. modulates myelopoiesis and enhances the resistance of tumor bearing mice. International Immuno Pharmacology. 2006; 6:294–299.
19. Jyothi TM, Shankariah MM, Prabhu S, Lakshminarasu S, Srinivasa GM, Setty SR. Hepatoprotective and antioxidant activity of *E. tirucalli*. Iran Journal Pharmacology. 2008;7:25-30.
20. Kane SR, Bhandari SV. *In vitro* phytochemical investigation, chracterization & antimicrobial study of latex of *Euphorbia tirucalli* Linn. International Journal of ChemTech Research. 2013;5(5):2344-2346.
21. Sales MDC, Costa HB, Fernandes PMB, Ventura JA, Meira DD. Antifungal activity of plant extracts with potential to control plant pathogens in pineapple. Asian Pacific Journal of Tropical Biomedicine. 2016;6(1): 26-31.
22. Jadhav DM, Gawai DV, Khillare EM. Evaluation of antibacterial and antifungal activity of *Euphorbia tirucalli.* Bionano Frontier. 2010;3(2):1-3.
23. Usman H, Abdulrahman FI, Usman A. Qualitative phytochemical screening and *in vitro* antimicrobial effects of methanol stem bark extract of *Ficus thonningii* (Moraceae). African Journal of Traditional, Complementary and Alternative Medicine. 2009;6(3):289–295.
24. Harborne JB. A guide to modern techniques of plant Analysis. USA: Kluwer Academic Publisher; 1998.
25. Skerget M, Kotnik P, Hadolin M, Rizner HA, Simonic M, Knez Z. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. Food Chemistry. 2005;89(2):191-198.
26. Wächtler B, Wilson D, Hube B. *Candida albicans* adhesion to and invasion and damage of vaginal epithelial cells: Stage-specific inhibition by clotrimazole and bifonazole. Antimicrobial Agents and Chemotherapy. 2011;55(9):4436-4439.

27. Kathiravan MK, Salake AB, Chothe AS, Dudhe PB, Watode RP, Mukta MS, Gadhwe S. The biology and chemistry of antifungal agents: A review. Bioorganic & Medicinal Chemistry. 2012;20(19):5678-5698.
28. Madane AN, Kamble SK, Patil BJ, Aparadh VT. Assessment of solvent solubility by using phytochemical screen tests of some Euphorbiaceae members. Asian Journal of Pharmacology Research. 2013;3(2):53-55.
29. Bhalodia NR, Shukla VJ. Antibacterial and antifungal activities from leaf extracts of *Cassia fistula* l.: An ethnomedicinal plant. Journal of Advanced Pharmaceutical Technology & Research. 2011;2(2):104-109.
30. Singh PS, Vidyasagar GM. Antifungal screening of 61 folkloric medicinal plant extracts against dermatophytic fungi *Trichophyton rubrum*. Journal of Applied Pharmaceutical Science. 2015;5(5):38-44.
31. Nonita PP, Mylene MU. Antioxidant and cytotoxic activities and phytochemical screening of four Philippine medicinal plants. Journal of Medicinal Plants Research. 2010;4:407–414.
32. Rajeh MAB, Zuraini Z, Sasidharan S, Latha LY, Amutha S. Assessment of *Euphorbia hirta* L. leaf, flower, stem and root extracts for their antibacterial and antifungal activity and brine shrimp lethality. Molecules. 2010;15(9):6008-6018.
33. Karimi E, Jaafar HZ, Ahmad S. Phytochemical analysis and antimicrobial activities of methanolic extracts of leaf, stem and root from different varieties of *Labisa pumila* Benth. Molecules. 2011;16(6):4438-4450.
34. Igbinosa OO, Igbinosa EO, Aiyegoro OA. Antimicrobial activity and phytochemical screening of stem bark extracts from *Jatropha curcas* (Linn). African Journal of Pharmacy and Pharmacology. 2009;3(2): 058-062.
35. Kamba AS, Hassan LG. Phytochemical screening and antimicrobial activities of Euphorbia balsamifera leaves, stems and root against some pathogenic micro-organisms. African Journal of Pharmacy and Pharmacology. 2010;4(9):645-652.

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