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Research Article

Evaluation of Antibacterial and Antimycotic Potency of *Euphorbia tirucalli* (L.) Growing Around Purano Bazar, Dharan, Sunsari, Nepal

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Abstract

Over thousands of years, people have used plants to heal a variety of ailments and infections. *Euphorbia tirucalli*, is a valuable member of the Euphobiaceae family, having a wide range of phytochemicals and potential applications in medicine. This in-vitro study was carried out to determine its efficacy against different types of disease and infection-causing bacteria and fungi. The plant's aerial portions were collected from Purano Bazar in Dharan City of Sunsari District in Province 1 of Nepal, and dried in a cabinet dryer at 30°C. The plant extracts were prepared using the Soxhlet apparatus and a range of solvents such as water, methanol, and chloroform. The plant extracts were collected and stored at 4°C for subsequent examination. The physiologically active components of *Euphorbia tirucalli* that provide antimicrobial properties were investigated. The total tannin, phenolic, and flavonoid contents were measured. The agar-well diffusion method was utilized to assess antibacterial and antifungal activity at different extract concentrations (0.3 and 0.6g/ml). Bacteria (*S. pyogenes, S. aureus, E. coli, S. typhi,* and *P. aeruginosa*) and fungi (*A. niger* and *C. albicans*) were used in the experiment. The lowest inhibitory concentration measured was 3.11mg/ml. The methanolic extract had effective antibacterial activity against *S. typhi,* with zones of inhibition (ZOI) of 21.5 and 33.16 mm. *Pseudomonas* had the lowest inhibition zone, measuring 12.83 mm and 17.17 mm. The aqueous and chloroform extract, on the other hand, showed no inhibition zones against fungi and bacteria. Only the methanol extract was effective against *Aspergillus niger* (ZOI: 11.19 mm and 9.5 mm) and *Candida albicans* (ZOI: 29.17 mm and 21.50 mm). No ZOI was found in chloroform and aqueous extracts. Variances in bioactive compounds and solvents could explain these variances in *Euphorbia tirucalli's* antimicrobial activity. The insights gained via further inquiry and analysis could be useful in the future for creating medications to combat different infections.

Keywords

Antimicrobial, Euphorbiaceae, Pharmacology, Methanol, Chloroform

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1. Introduction

Plants are the richest sources of Botanical medicines with antimicrobial efficiency in the world; one such miracle plant is *Euphorbia tirucalli*. It is commonly known as the pencil cactus, milk bush, firestick or tetlung plant. Pencil cactus is one of the most valuable plants known worldwide having multiple uses. Pencil cactus is a member of Euphorbiaceae family [1]. It is a succulent plant indigenous to tropical and subtropical regions, including Asia and Africa. The area around Purano Bazar, Dharan, Sunsari in Nepal is well-known for its affluent biodiversity, including numerous medicinal plants used by local communities. As part of traditional curative practices, local inhabitants have used *Euphorbia tirucalli* to treat wounds, skin diseases, and infections [2]. The plant's role in traditional medicine is well-documented in ethnobotanical studies, where it is often employed in poultices and topical applications. It is planted as a hedge plant in gardens and cultivated fields [3]. Due to its resilience to severe aridity stress, the pencil cactus is one of the most significant plants, typically planted for boundary delineation but also serving as a living fence surrounding complexes, shrines, and kraals [4].

Conventionally, it has been used in folk medicine for various ailments, including inflammatory conditions, and skin disorders, and even as a remedy for infections. Because of its proteolytic, molluscidal, and larvicidal properties, *Euphorbia tirucalli* has been used extensively in traditional medicine to treat syphilis, as well as to treat skin tumours, cancer, epithelioma, sarcoma, cough, asthma, and rheumatism [5] (Swapna et al., 2020). Many researchers have recently shown interest in natural plant extracts owing to their impulsive or volatile nature, flavour, antioxidant, and antimicrobial effects [6, 7] (Dong et al., 2020; Topa et al., 2020). Numerous medicinal plants are being screened for potential antibacterial action due to the antibiotic resistance displayed by harmful contagious pathogens [8, 9] (Kumar et al. 2015; Garcia et al., 2024). In particular, extracts from various parts of the plant—such as leaves, stems, and latex—have been shown to exhibit bioactive compounds capable of inhibiting the growth of a range of pathogenic microorganisms. The antimicrobial properties of *Euphorbia tirucalli* have been fundamentally attributed to its rich phytochemical profile, including alkaloids, flavonoids, saponins, terpenoids, and phenolic compounds which contribut to medicinal treatment effectively [10]). Alkaloids, flavonoids, terpenoids, polyphenols, and phenols are all produced through secondary metabolism in plants[11, 12]. (Gulcin et al., 2004; Abad et al., 2007).

These compounds are known for their antibacterial and antifungal activities. The plant's latex, which contains a variety of bioactive compounds, has shown particular promise in combating microbial infections. This plant may have therapeutic qualities and minimal herbivore stress due to the white poisonous latex it secretes. Alfaeuforbol, taraxa, alcohol eufol, and tirucallol are among the identified compounds found in the plant extract, along with massive levels of sterols and terpenes [13]. (Gupta et al., 2012). Furthermore, it has been noted that *E. tirucalli's* latex and other plant parts exhibit pesticity in attempts to investigate its potential as a medicine. The plant has little herbivore pressure due to its white toxic latex [4].(Sultan et al., 2016). Additionally, there have been reports of the pesticidal effects of *E. tirucalli's* latex and other plant components against pests such aphids (*Brevicoryne brassicae*) and mosquitoes *Aedes aegypti* and *Culex quinquefasciatus* in attempts to investigate the plant's potential for therapeutic use [14, 15]. (Tiwari & Singh, 2006; Mwine & Van Damme, 2010). Some studies have isolated specific compounds that exhibit both broad-spectrum and selective antimicrobial properties, making it a potential candidate for the development of alternative therapeutics. Furthermore, studies have shown the herb's antioxidant capacity, which suggests that it may have anticancer properties [16].(Munro et al., 2015). Pencil cactus has larvicidal, anticancer, antibacterial, herpatoprotective and many other properties, thus it has become the center of biochemical researches [17]. Given the growing interest in the antimicrobial properties of local plants in Nepal, this study aims to explore the efficacy of *Euphorbia tirucalli* in the region of Dharan.

2. Materials and Methods

2.1. Study Area

Euphorbia tirucalli's aerial parts were gathered in Purano Bazar, Dharan. Longitude: 87°17.242'E, latitude: 26°48.940'N, elevation: 382 meters above mean sea level. The study was carried out in a laboratory at the Central Campus of Technology in Province No. 1 in the Sunsari district of the Dharan region of Nepal. Figure 1 depicts the location of the study region.

2.2. Plant Sample Collection

The stems and leaves were manually picked from healthy, sturdy plants and promptly transported to the CCT laboratory in Dharan for drying within half an hour of collection from the study site.

2.3. Preparation of Plant Crude Extracts

The extract from 10g of the sample was prepared using Soxhlet apparatus using a solvent extraction technique, which included 250ml of polar solvents methanol, distilled water, and non-polar solvent chloroform. The extract was kept at 4 ℃ until the qualitative and quantitative assays were done [18].

2.4. Microbial Strains

The strains of microorganisms required for the study were obtained from the Department of Microbiology, CCT, Dharan. The test organisms taken were gram positive bacteria *Staphylococcus aureus* (ATCC 25923), *Streptococcus pyogenes*, gram negative bacteria like *Pseudomonas aeroginosa*, *Salmonella typhi*, *and Escherichia coli* (ATCC 25922) and fungal strains used in the study for antimycotic evaluation were *Aspergillus niger* and *Candida albicans*. Bacteria were subcultured in Mueller Hinton Agar (MHA) nutrient broth for 24 hours at 37 ℃, whereas fungal strains were subcultured on Sabouraud dextrose agar at 37 ℃ for 24 hours [19, 4].

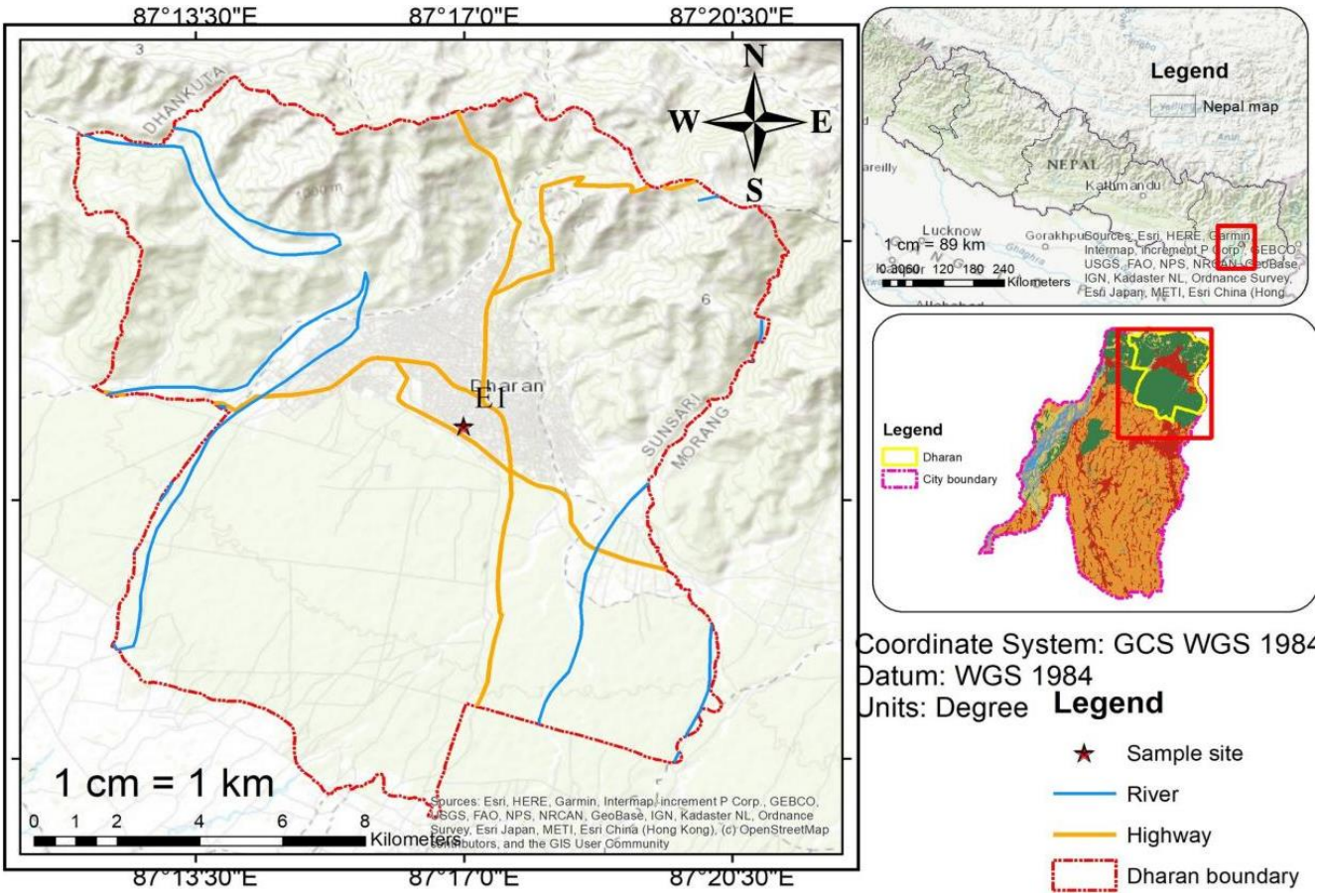

Figure 1. *Map of study area.*

2.5. Screening of Phytoconstituents of Plant Extracts

The plant crude extracts underwent qualitative phytochemical analysis using established techniques for such tests [20]. with slight modifications.

The solvent extracts were analyzed for alkaloids, steroids, proteins, flavonoids, carbohydrates, saponins, quinines, anthraquinones, tannins, phenolic compounds, oils, and glycosides [18].

2.6. Estimation of Total Phenolic Content (TPC)

The Folin-Ciocalteu technique was used to determine total phenol content. A test tube was filled with 0.5ml of extract and 4.5ml of distilled water. After shaking the mixture thoroughly, 0.5ml of Folin-Ciocalteu phenol reagent was added. Five minutes later, 5ml of 7% sodium m carbonate solution was added to the mixture. Gallic acid standard solutions were prepared at the following concentrations: 10, 20, 40, 60, 80, and 100g/ml. After incubating the mixture for 30 minutes at room temperature, an ultraviolet (UV) or visible spectrophotometer was used to measure the absorbance of the test and standard solutions at 765nm vs. the blank reagent [21].

2.7. Estimation of Total Tannin Content (TTC)

For determination of total tannin content the Folin-Ciocalteu technique was used. About 0.1ml of sample extract was added to a test tube containing 7.5ml of distilled water and 0.5ml of the Folin-Ciocalteu phenolic reagent. Following that, 1ml of a 35% Na_2CO_3 solution was incorporated, followed by 10ml of distilled water to dilute it. The mixture was

kept at the ambient temperature and then shook vigorously. Gallic acid standardized reference solutions wi th concentrations of 10, 20, 40, 60, 80, and 100g/ml were prepared. The absorbance of test and reference solutions at 725nm was measured in comparison to the blank using a UV/Visible spectrophotometer. The tannin percentage was estimated employing mg of GAE/g of extract [22].

2.8. Estimation of Total Flavonoid Content (TFC)

The total flavonoid content (mg/mL) was calculated using the aluminum chloride ($AlCl_3$) procedure. A test tube had 0.3ml of 5% sodium nitrite, 0.5ml of distilled water, and 0.5ml of plant extract. The assay mixture was kept at room temperature for 5 minutes. Following incubation, 0.3ml of 10% aluminium chloride was administered immediately. The mixture was combined with 2ml of sodium hydroxide, and the absorbance was measured at 415nm. Quercetin served as a standard [23].

2.9. Evaluation of Antibacterial and Antimycotic Activity of Crude Extracts of *Euphorbia tirucalli*

Mueller Hinton Agar (MHA) plates were employed for the agar well diffusion technique for antimicrobial testing of plant extracts [24, 25]. To achieve a turbidity of 0.5 McFarland standards, the test organisms were injected in Nutrient broth and cultivated overnight at 37 ℃, providing a final inoculum of 1.5 108 CFU/ml. The MHA plate was lawn cultivated using standardized microbial culture broths. The plant extracts at a concentration of 100mg/ml were prepared using Dimethyl Sulfoxide (DMSO). The procedures followed for the antimycotic assays were described by Niño et al. (2012). 6 mm wells were drilled into the contaminated medium using a sterilized cork-borer. For the evaluation of antimicrobial efficacy different concentrations of extract were used. Each well was filled with 0.6g/ml and 0.3g/ml extracts of plant, antibiotic discs were used for positive control and extract for negative/solvent control. For each specimen, the experimental process was repeated three times. During every experiment, readings were taken from three distinct fixed orientations, and the average findings were recorded. It was incubated at 37 ℃ for 24 hours after being allowed to diffuse for 30 minutes at ambient temperature [26, 27]. Following incubation, the antibacterial and antifungal activity of the test compounds were assessed by inspecting the plates for the formation of a clear zone around the well. Inhibitory zones (ZOI) were measured in millimeters.

2.10. Minimum Inhibitory Concentration of *Euphorbia tirucalli* Extract

The minimum inhibitory concentration (MIC) is commonly used to evaluate the efficacy of antimicrobial drugs against certain infections. In this study, the MIC values of *Euphorbia tirucalli* extracts were determined using a 96-well titer plate. To attain different concentrations, the test extracts are diluted in a sterile medium for the 96-well titer plate method. Incubate the plates at the appropriate temperature for the fungal and bacterial strain being tested. Add 5µl of bacterial suspension to each well containing the diluted extract of 50µl with 45µl of nutrient broth. Following incubation, the MIC is calculated by determining the extract's lowest dose at which bacterial growth is inhibited. The MIC values determined using this technique provide a numerical assessment of the extracts' efficacy against the test bacteria and fungi. The extract's antibacterial and antifungal activity against the bacterium and fungus under test becomes stronger as the MIC value decreases; it was the lowest concentration of plant extract where no microbial growth and turbility was seen [28].

The MIC was the lowest concentration of extract with no visible bacterial growth or no turbidity.

3. Results and Discussion

3.1. Screening of Phyto-constituents Using Different Solvents

The screening of crude extracts of *Euphorbia tirucalli* showed the presence of different bioactive compounds. The phytochemicals obtained in different solvents are listed on table 1, the obtained results showed the presence of phenols, flavonoids, tannins, oils, carbohydrates, amino acids, and glycosides. The findings of phytochemical screening revealed that, the variation of compounds are highly influenced by the type of solvents and methods used. Methanol as a solvent was found most effective than chloroform and distilled water. The polarity and molecular weight of the solvent used may influence the extraction of phytochemical components [29]. The previous studies related with the phytochemical screening of *Euphorbia tirucalli* extract revealed the presence of different phytoconstituents like alkaloids, flavanoids, tannins, phenols, steroids, saponins, diterpenes, and cardiac glycosides [4].

Table 1. Qualitative evaluation of phytochemicals for Euphorbia tirucalli in different solvents.

S.N	Phytochemicals	Methanol extract	Aqueous extract	Chloroform extract
1	Phenols	+	+	_
2	Terpenoids	_	_	_
3	Tannins	+	+	_
4	Amino acid	+	+	+
5	Glycosides	+	_	_
6	Flavonoids	+	+	+
7	Carbohydrates	+	_	+
8	Oils	+	_	+
9	Alkaloids	_	_	_
10	Quinones	_	_	_
11	Anthraquinone	_	_	_
12	Saponins	_	_	_

*The "+" sign represents the presence of phytochemicals, and the "-" sign represents the absence of phytochemicals

3.2. Quantitative Evaluation of Biologically Active Compounds in *Euphorbia tirucalli*

The quantitative analysis of the crude extract of *Euphorbia tirucalli* was done to determine total phenolic content (TPC), total tannin content (TTC), and total flavanoid content (TFC) in different solvents (Table 2). TPC of the plant extract in methanol, aqueous and chloroform were 14.20mgGAE/g, 134.20 mgGAE and 31.35 mgG/g. Similarly, TTC were also recorded 104.65mgGAE/g, 66.29mgGAE/g and 13.57mgGAE/g. TFC at were 354.93 mgQuercetin/g, 84.20mgGAE/g and 3.52 mgQuercetin/g. The phenolic content was highest in aqueous, then in methanol and least in chloroform. Both Total tannin content and total flavanoid content recorded were highest in methanol and least in chloroform. It may be because some compounds are not soluble in chloroform and water [30].

It is also possible that compound may not soluble in petroleum ether, chloroform, methanol and water.

3.3. Antimicrobial Susceptibility Test of *Euphorbia tirucalli* Extract

Antibacterial and antimycobiont efficacy was evaluated by measuring the zones of inhibitions (ZOI) in mm (Table 3). At 0.3g/ml concentration methanol extract showed the highest antimicrobial activity on *S. typhi* with ZOI of 21.50±2.00 and lowest in *E. co li* with ZOI 12.67±0.28. At 0.6g/ml concentration the strongest inhibition was seen in *S. typhy* measuring ZOI 33.17 2.08 and weakest activity was observed in *P. aeruginosa* 17.17±0.57. Antifungal activity at 0.3g/ml concentration was highly effective in *candida albicans* with ZOI 21.50 ±3.96 and was found less effective in *Aspergillus niger* showing ZOI of 9.50±1.32 only. Similarly at 0.6g/ml concentration methanol extract had shown stronger inhibition against *Candida albicans* (ZOI 29.17±4.07) and was found weaker in *Aspergillus niger* (ZOI 11.19±0.57). However the aqueous and chloroform extracts were ineffective against the test pathogens. The methanol extract had the strongest effectiveness against gram-positive bacteria and fungus as well. *E. tirucalli* has the best antibacterial action [31].

The presence of diverse phytocompounds in varying levels in plant extracts may have contributed to the antibacterial activity investigated in this study (Table 1). The tested antimicrobial activity of plant species is determined by the botanical species, age, section of the plant investigated, and solvent utilized in the extraction techniques [32].

Table 2. Total phenolic content, total tannin content and total flavonoid content of Euphorbia tirucalli in different wavelengths using different solvents.

Phy tochemicals	Methanol extract	Aqueous extract	Chloroform xtract
Total phenolic content (mg GAE/g dry matter) / at 765nm	134.20	156.58	31.35
Total tannin content (mg GAE/g dry matter) / at 725nm	104.65	66.29	13.57
Total flavonoid content (mg Quercetin/g dry matter) / at 415nm	354.93	84.20	3.52

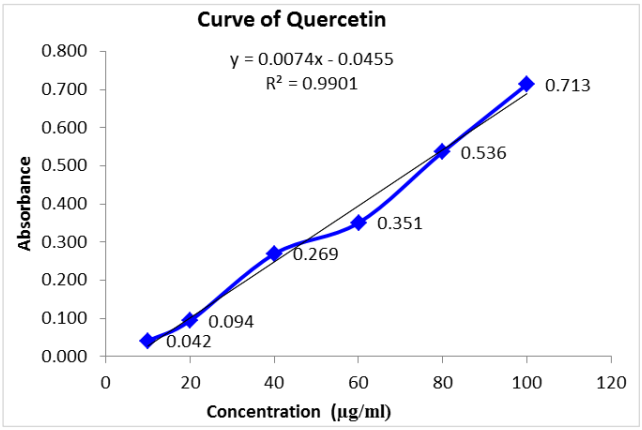

Figure 2. Calibration curve of Quercetin.

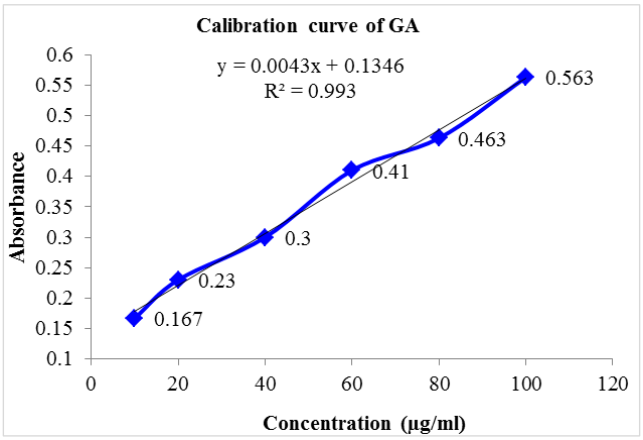

Figure 3. Calibration curve of Gallic acid.

Table 3. Antimicrobial Activity of plant extracts.

Microorganisms	Zone of inhibition in Methanol extract		
	0.3 (g/ml)	0.6 (g/ml)	Tetracycline (g/ml)
1. *S. aureus*	14.83±0.57	19.16±1.60	34
2. *E. coli*	12.67±0.28	18.50±0.50	22
3. *S. pyogenes*	13.17±1.52	20.18±2.56	35
4. *P aeruginosa*	12.83±0.76	17.17±0.57	23
5. *S typhy*	21.50±2.00	33.17±2.08	28
6. *A. niger*	9.50±1.32	11.19±0.57	39.5
7. *C. albicans*	21.50±3.90	29.17±4.07	37

3.4. Minimum Inhibitory Concentration (MIC) of Plant Extracts

Table 4 displays the results for the minimum inhibitory concentration of *Euphorbia tirucalli* extracts prepared in different solvents. Methanol extracts scored higher for anti-bacterial and antifungal activity than other solvents, with minimum inhibitory concentrations ranging from 3.11mg/ml to 7.50mg/ml. A broad spectrum antibiotic Tetracycline had MIC value of 1.03mg/ml (Table 4).

Table 4. Minimum Inhibitory Concentration of plant extract in different solvents.

Microorganisms	Solvents used		MIC value	
	Aqueous (mg/ml)	Chloroform (mg/ml)	Methanol (mg/ml)	Tetracycline (mg/ml)
1. *S. aureus*	-	-	4.51	2.5
2. *E. coli*	-	-	5.10	3.05
3. *S. pyogenes*	-	-	4.22	2.04
4. *P aeruginosa*	-	-	6.32	55.31
5. *S typhi*	-	-	3.11	5.00
6. *A. niger*	-	-	7.50	1.12
7. *C. albicans*	-	-	3.45	1.03

Table 5. Kruskal-Wallis test for equal medians.

Test for equal means					
Souce	Sum of Squares	df	Mean square	F	p (same)
Between groups	91.7504	1	91.7504	0.455	0.5128
Within groups	2419.96	12	201.663 (n=99999)	Permutation p	
Total	2511.71	13	0.9927		
Components of variance (only for random effects)					
Var (group)	-15.7019	Var (error)	201.663	ICC	-0.08444
Omega 2	0				
Levene´s test for homogeneity of variance, from means			p (same)	0.05162	
Levene´s test, from medians			p (same)	0.3446	
Welch F test in the case of unequal variances: F=0.455, df=6.074, p=0.5248					

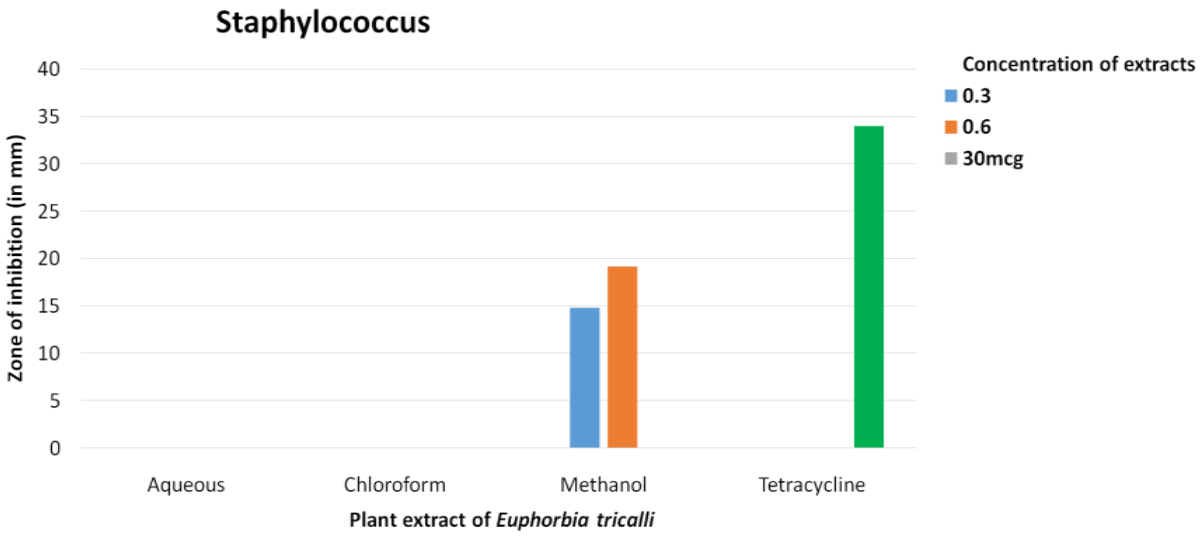

Figure 4. Antibacterial activity of Euphorbia tirucalli extract against S. aureus.

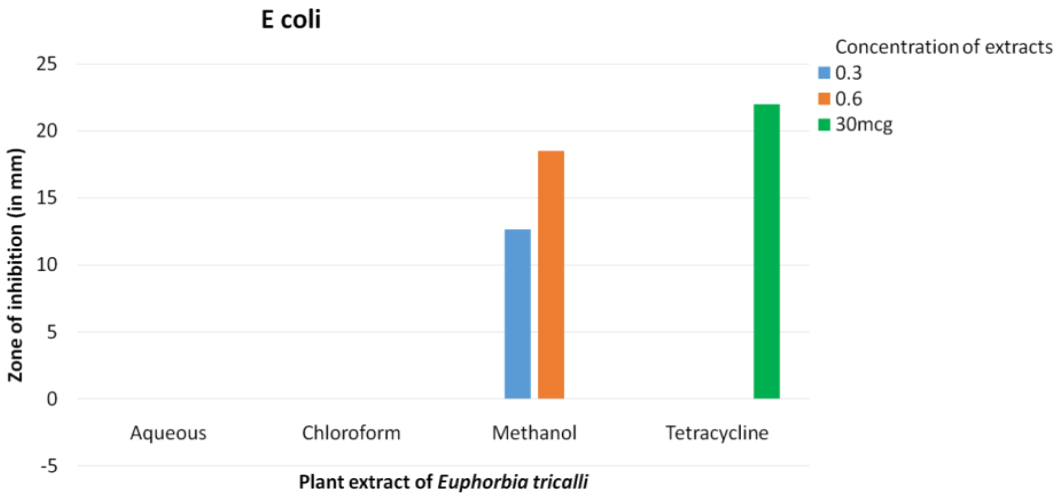

Figure 5. Antibacterial activity of Euphorbia tirucalli extract against E. coli.

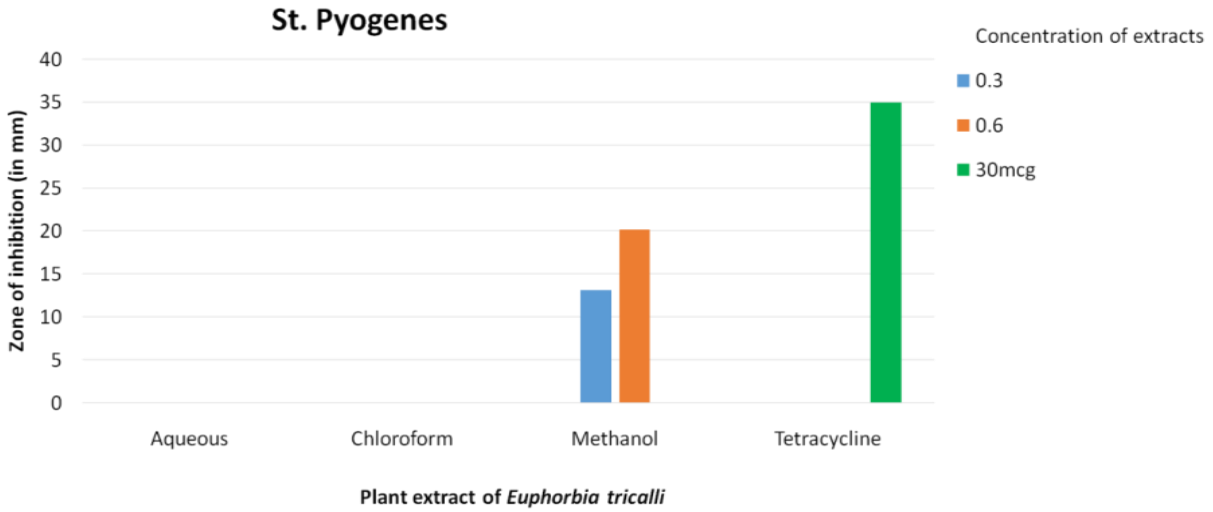

Figure 6. Antibacterial activity of Euphorbia tirucalli extract against S. pyogenes.

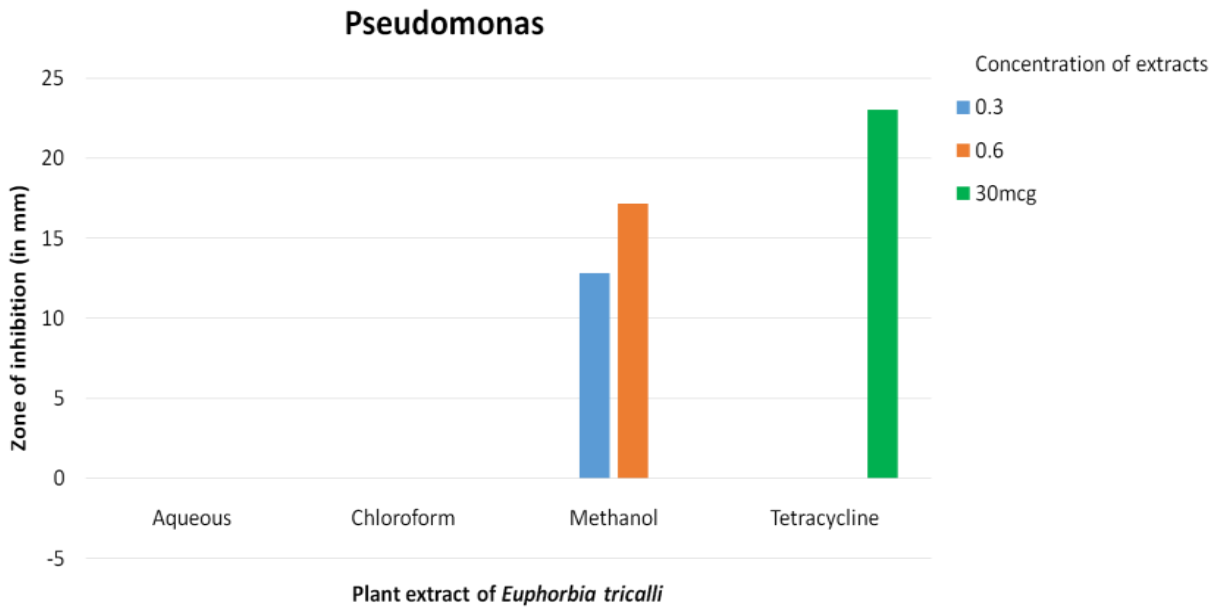

Figure 7. Antibacterial activity of Euphorbia tirucalli extract against P. aeruginosa.

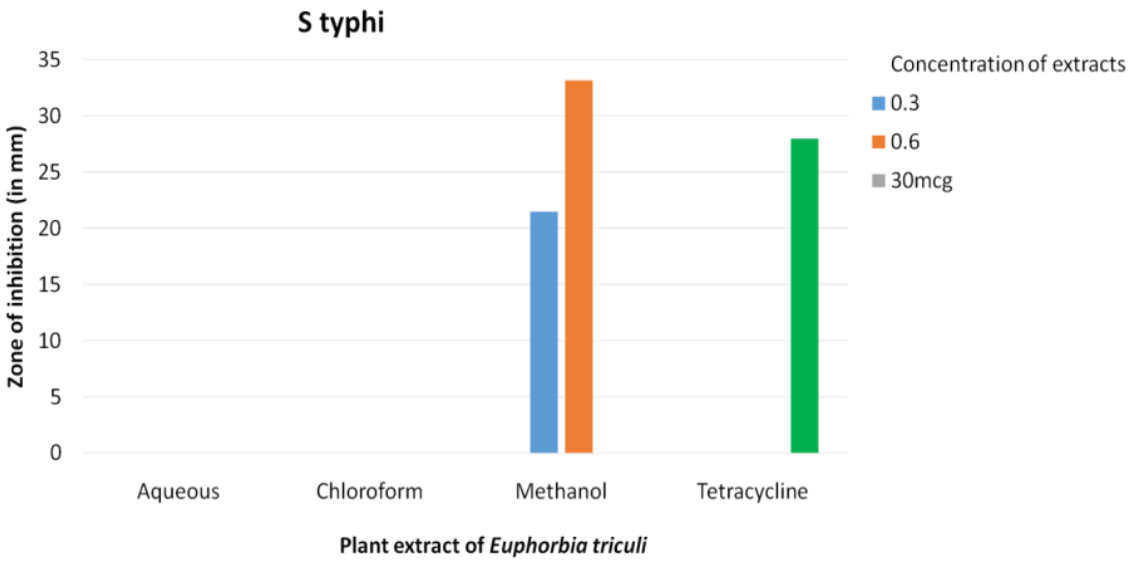

Figure 8. Antibacterial activity of Euphorbia tirucalli extract against S. typhi.

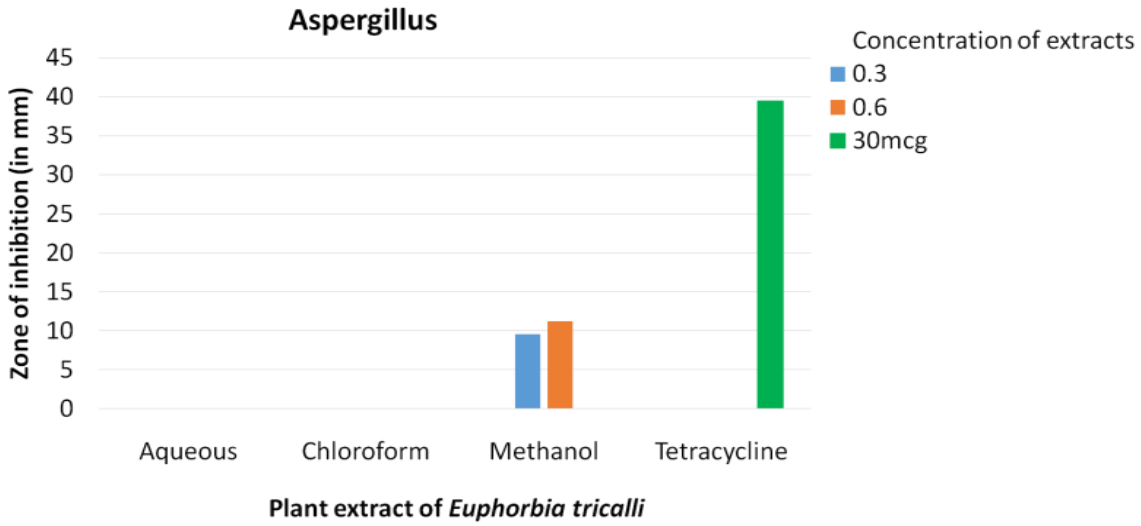

Figure 9. Antifungal activity of Euphorbia tirucalli extract against Aspergillus niger.

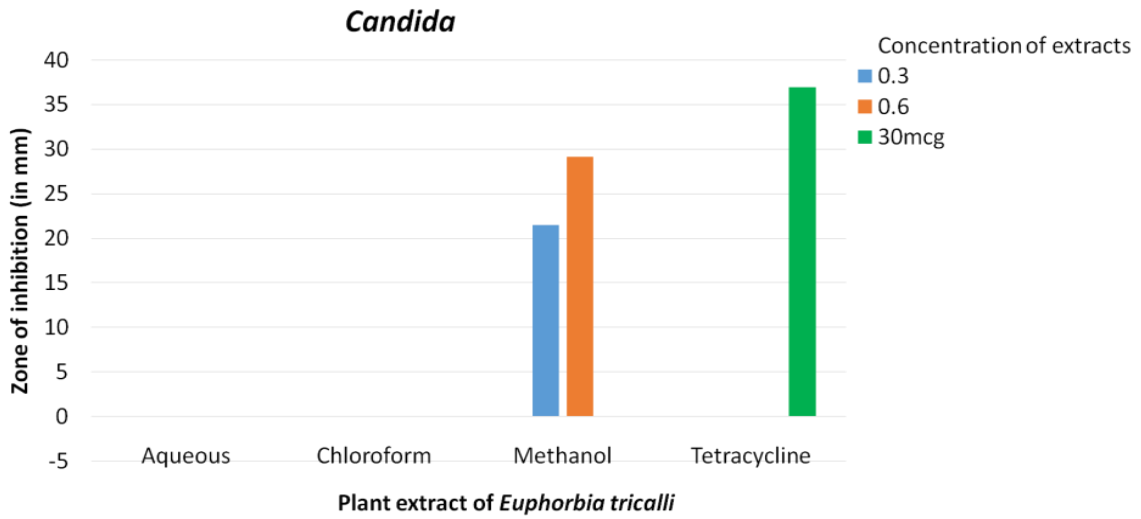

Figure 10. Antifungal activity of Euphorbia tirucalli extract against Candida albicans.

4. Conclusion

This study has revealed that the extract of *Euphorbia tirucalli* has numerous biologically active phytochemicals like glycosides, flavonoids, amino acids, carbohydrates, tannins, oils and phenols. The results obtained demonstrated the effective activity of plant extract against different strains of bacteria and fungi. The most effective level of antibacterial activity was noticed on *S. typhi* and least on *E. coli.* Similarly the antifungal activity showed highest level of action on *Aspergillus niger* and lowest level in *Candida albicans*. Additionally, the obtained result demonstrated that the methanol was determined to be the best solvent for phytochemical screening and antimicrobial susceptibility test. Thus, *Euphorbia tirucalli* plant can be used for therapeutic purposes.

Acknowledgments

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Conflicts of Interest

The authors declare no conflicts of interest.

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