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Research Article

Antimicrobial Activities of *Euphorbia Hypericifolia* L. and Testing of its Anti-Enteric Effect

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Abstract

Medicinal plant *Euphorbia hypericifolia* L. reputed to possess the curative power in enteric infections was selected, tested and evaluated for their activities by *in vitro* technique. *Euphorbia hypericifolia* L. was extracted with 50% ethanol, 95% ethanol, ethyl acetate and aqueous solutions and extracts were used to screen enteric infections. These extracts were found to be potent. Antibacterial activities of its plant was tested, According to the results, the extracts made by organic solvents revealed the distinct antibacterial activity on gram-negative bacteria such as *E.coli, Salmonella, Shigella,* and *Vibrio.* It may be used as a remedy for the treatment of dysenteric patients but we need large randomized control trials to confirm its effect in human model.

Introduction

Euphorbia hypericifolia L. belongs to the family Euphorbiaceae. Euphorbiaceae is a large family of 283 genera with about 7300 species, of almost cosmopolitan distribution (Laurence, 1969), mainly tropical [1]

but extending also to temperate regions of both the Northern and Southern hemispheres [2]. The outstanding features of the family Euphorbiaceae are the presence of latex, conspicuous unisexual flowers and tricarpellary the subtropical and warm temperate regions. It grows wide abundantly in Myanmar. In the British Pharmaceutical Codex (1954) *Euphorbia hypericifolia* L. is said to contain water soluble constituents, gallic acid, quercetin, phenolic substances, an amorphous glycoside and a sugar. The portion soluble in alcohol, but insoluble in water, contains a monohydric alcohol, euphosterol, a phytosterol and phytosterolin, jambulol, melissic acid and a mixture of fatty acid [2]. The leaves are eaten as a vegetable and contain 78.14% moisture, protein, 4.65%; ash 3.15% and vitamin C, 44.32mg/100g. The latex of this plant is used as an application for warts.

Matindale (1967) stated that a tincture of *Euphorbia hypericifolia* L. has been recommended since 1932 in the treatment of cough and asthma. In British Herbal Pharmacopoeia (1976), it is prescribed for the treatment of asthma, bronchitis, upper respiratory catarrh, and intestinal amoebiasis. In Malaysia, *E. hypericifolia* L. has various medicinal uses [3]. The latex is used as eye drop for conjunctivitis and complaints of the eye. It is also used for poulticing sores on the legs and for bruises, and wounds due to the marine worm and cholera. The Philipinos also use it as a poultice. Chinese herbalists also use *Euphorbia hypericifolia* L. Bacteria are prokaryotic cells whose single chromosome is not contained within a nuclear membrane. The bacterial cytoplasm is surrounded by a plasma membrane (Nicklin, Graeme-Cook, Paget & Killington, 1999). Some bacteria are variable in shape and are said to be pleomorphic (of many shapes). The shape of a bacterium is determined by its rigid cell wall. The size of bacteria is in the range from 0.2-50urn. They show little structural differentiation when examined by ordinary microscopic methods, but special staining method show that they possess a central nuclear body which contains deoxyribonucleic acid and divides by simple fission without evidence of mitosis or chromosomes. Types of bacterial organism were expressed in Table 1.

Table 1: *Types of bacteria and occurrence [4]*

Type of Bacteria	Occurrence
Escherichia coli	Diarrhoea, haemorrhagic diarrhoea, dysentery, pyelitis, cystitis, appendix abscess, peritonitis, cholecystitis, septic wounds & bed sores, gastroenteritis
Shigella	Acute dysentery, bloody diarrhoea, bacillary, dysentery, bacteraemia
Salmonella	Necrosis, typhoid, food poisoning, acute suppurative periosteitis, osteitis, abscess of the kidney, acute cholecystis, bronchopneumonia, empyema, ulcerative endocarditis
Vibrio	*Vibrio cholerae* typical cholera, other species can cause food poisoning, diarrhoea, tenesmus, vomiting
Bacillus subtilis	Saprophytes, usually non-pathogenic
Staphylococcus aureus	Nosocomial infections, hospital acquired infections, food poisoning, boils, abscesses, wound sepsis

Materials and Methods

Bacteria Used in the Experiments

The bacteria species used were obtained from the Department of Medical Research (Lower Myanmar), Yangon, and listed in Table 1.

Chemicals and Reagents

Trypticase soy broth from Difco, trypticase soy agar from Becton, and triple sugar iron agar from Difco, Ethyl acetate, 95% ethanol, 50% ethanol, and distilled water were used. The chemicals used were from British Drug House (B.D.H Chemical Co. Ltd., (England), Kanto Chemical Ltd., (Japan) and Merck Co., Ltd., (Germany). Most of the chemicals and reagents employed in this study were from B.D.H Chemical Co. Ltd., England", Sigma Chemicals Co., St. Louis, U.S.A", Difco Laboratories, (Detroit, U.S.A), E. Merck, (Darmstadt, Germany) and Becton Dickson and Co., (Cockeysville, U.S.A).

Apparatus

Conical flasks, petridishes, test tubes, measuring cylinders, a constant temperature bath (Yamato Scientific Co., Ltd. Japan), a hot air sterilizer (Tomy seiko Co., Ltd., Tokyo, Japan), a refrigerator (National Co., Ltd., Japan), Wire loops, straight wire, spirit burner and aluminium foil were employed.

Preparation of Crude Extracts of *Euphorbia hypericifolia* L.

The air dried fine powder (100)g was soaked in 500ml of 50% ethanol, 95% ethanol, ethyl acetate and distilled water for about 2 days and then filtered. By using a rotary evaporator the solvents were then allowed to evaporate so as to obtain a paste or powder form. Then the dried extracts were weighed.

Determination of Antibacterial Activity of Various Crude Extracts of *Euphorbia hyperici folia* L.

For the determination of antibacterial activity of crude extracts *in vitro*, agar disc diffusion method was used because of its simplicity, speed of performance, economy and reproducibility.

Preparation of Agar Medium

Trypticase soy agar (40)g was suspended in 1000ml of distilled water in a sterile conical flask and covered with aluminium foil. Then the suspension was mixed thoroughly and heated completely to dissolve the powder on a hot plate stirrer [4]. This Trypticase was then sterilized in an autoclave at 121˚C for 15mins. The temperature of the agar solution was reduced to 50˚C in a constant temperature bath. Trypticase soy agar was then poured into sterile petridishes near a flame of a spirit burner. The agar medium was allowed to solidify and sealed tightly in a polythene plastic bag. The medium was stored in a refrigerator until used. The solidified agar medium was dried in 42˚C incubator before it was used.

Preparation of Agar Slant Medium

Triple sugar iron agar (65g) was suspended in 1000ml of distilled water in a sterile conical flask, covered with aluminium foil and mixed thoroughly and heated completely to dissolve the powder on a hot plate stirrer. Then triple sugar iron agar solution was transferred into test tubes (4ml for each) and sterilized by autoclaving at 121˚C for 15mins. After sterilization, the test tubes were placed in a slant position and allowed to solidify.

Preparation of Broth Medium

Trypticase soy broth (30g) was suspended in 1000ml of distilled water in a conical flask, covered with aluminium foil, mixed thoroughly and heated on a hot plate with magnetic stirrer till dissolved [4,5]. The broth solution was then transferred into test tubes (3ml in each tube) and sterilized by autoclaving for 15mins at 121˚C.

Bacterial Culture

The bacteria species used were obtained from the Department of Medical Research (Lower Myanmar), Yangon, and were listed in Table. A few colonies of the organism to be tested were inoculated into the triple sugar iron agar and incubated at 37˚C for 24hrs in an incubator. A few colonies of the organism from triple sugar iron agar were introduced into the trypticase soy broth and incubated for 3hrs at 37˚C to obtain the bacterial suspension of moderate cloudiness. This contained approximately 10^5 to 10^7 organisms per ml.

Testing by Agar Disk Diffusion Method

Water, 50% ethanol, 95% ethanol and ethyl acetate extracts of *Euphorbia hypericifolia* L. were prepared. The bacteria species tested were the same as those given in Table 2.

Table 2: *Organisms and their respective Code Numbers.*

No.	Organisms	Code
1	*Bacillus subtilis*	DMR-BS
2	*Klebsiella aerogenes*	NCTC-418
3	*Proteus morganii*	BIKEN
4	*Pseudomonas pyocyanea*	DMR-004
5	*Escherichia coli ETEC*	DMR-H 1-3.
6	*Escherichia coli LT*	DMR-536-2
7	*Escherichia coli EAEC*	DMR-N 10/83
8	*Escherichia coli EHEC*	DMR-Su 10-1
9	*Escherichia coli ATCC*	ATCC-25922
10	*Escherichia coli EPEC*	DMR-WT 87

11	*Salmonella paratyphi*	DMR-WT 684
12	*Salmonella Stanley*	DMR-WT 346
13	*Salmonella spp*	DMR-chick
14	*Salmonella typhi*	DMR-ID-3, BIKEN
15	*Salmonella typhi*	DMR-sep 110
16	*Plesiomona shigelloides*	DMR-WT 8
17	*Shigella boydii*	DMR-N 136-7
18	*Shigella boydii*	DMR-N 284-2
19	*Shigella dysenleriae*	DMR -sd 4
20	*Shigella flexneri*	DMR-No 113-2
21	*Shigella flexneri*	DMR-N08I
22	*Shigella sonnei*	DMR- No 398-2
23	*Shigella sonnei*	DMR-A 281-6
24	*Shigella sonnei*	DMR-SS
25	*Staphylococcus aureus*	DMR-Pa-3
26	*Staphylococcus aureus*	DMR-Sa-3
27	*Staphylococcus aureus*	DMR-Sa-4
28	*Staphylococcus aureus*	DMR-Sa-54
29	*Staphylococcus aureus*	DMR-Sa-83
30	*Staphylococcus aureus*	DMR-Sa-96
31	*Staphylococcus aureus*	DMR-Sa-220
32	*Staphylococcus aureus*	DMR-Sa-
33	*Vibrio cholerae Inaba*	DMR-KSA
34	*Vibrio cholerae 0139*	DMR-Vc 48
35	*Vibrio parahaemolyticus*	BIK- 1387

Preparation of Sample for Testing

The plant extract (0.2g each) was introduced into sterile petri-dishes and dissolved with 1ml of their respective solvents viz., 50% ethanol, 95% ethanol, ethyl acetate and distilled water. The antibacterial activity of the extracts was determined by the agar disc diffusion technique. Screening was done by the use of impregnated paper-discs (8mm) which were sterilized by autoclaving, followed by dry heat at 60°C for 1hr. It was then impregnated with concentrated extracts and allowed to dry at 42°C in an air flow incubator. The bacterial suspension from Trypticase soy broth was streaked evenly onto the surface of the Trypticase soy agar plates with a sterile cotton swab (Puriton, USA). After the inoculum had dried (5mins), the dried discs were placed on the agar with flamed forceps, gently pressed down to ensure proper contact. A disc impregnated with solvent only was placed along the side of test discs for control and comparing purposes.

The plates were incubated immediately (or within 30mins) after inoculation. After overnight incubation at 37°C, the zones of inhibition diameter (including the 8mm discs) were measured.

Determination of Minimum Inhibitory Concentration (MIC) of the Active Extracts by Plate Dilution Method

In order to determine the minimum concentration of extracts that inhibit the microorganism, the specific concentrations of extracts prepared in serial dilution in agar plates were used for testing with microorganisms. Fifty percent ethanol extract of *Euphorbia hypericifolia* L. was selected for determination of MIC. The minimum inhibitory concentration (MIC) of the extracts was determined by test tube serial dilution method [4,5].

The active plant extracts were dissolved with their respective solvents (e.g., ethanolic extracts with ethanol) and diluted with trypticase agar to obtain the following concentrations (e.g. 0.5g) of the plant extracts was dissolved in 2ml of solvent and diluted with 40ml of trypticase soy agar, where the concentration will be from MIC 0.625mcg/ml to 1mcg/ml. The above solutions of varying concentrations were each poured into sterile petridishes. Trypticase soy agar containing only the solvent was also prepared with sterile petridish for control purposes. Then the agar medium were allowed to solidify.

Bacterial suspension was obtained by inoculation of a few colonies of the organisms to trypticase soy broth and incubated at 37°C for 3hrs. The bacterial suspension was streaked onto the surface of the prepared agar plates. Then the plates were incubated at 37°C overnight. After this, the lowest concentration showing no growth of the organisms was taken as the minimum inhibitory concentration (MIC) expressed in mg/ml. The experiments were repeated three times at exactly the same parameters, and the mean results were taken.

Results

Antibacterial Activity of Different Extracts of *Euphorbia hyperleifolia* L. Screening by Agar Disc Diffusion Method

The antibacterial activity of *Euphorbia hypericifolia* L. extracts by different solvents were estimated by agar disc diffusion method of Ananthanaragan & Paniker (1983). In this test 35 species of bacteria, including eight species of Shigella, eight species of Staphylococcus aureus, six species of *Escherichia coli*, five species of *Salmonella*, three species of *Vibrio*, a species each of *Bacillus subtilis, Klebsiella aerogenes, Proteus morganii* and *Pseudomonas pyocyanea*, were used as test organisms (Table 3).

Table 3: *Antibacterial activity of Euphorbia hypericifolia L. on 35 species of bacteria (mean zone diameter in mm).*

No.	Organisms	Extracts			
		50% ethanol	95% ethanol	ethyl acetate	water
1	*Bacillus subtilis*	14	-	-	-
2	*Klebsiella aerogenes*	-	-	-	-

3	*Proteus morganii*	12	-	-	-
4	*Pseudomonas pyocyanea*	19	14	-	14
5	*Escherichia coli ETEC*	23	20	1 5	-
6	*Escherichia coli LT*	20	-	-	-
7	*Escherichia coli EHEC*	16	-	-	-
8	*Escherichia coli EHEC*	-	-	-	17
9	*Escherichia coli ATCC*	18	-	-	-
10	*Escherichia coli EPEC*	-	-	-	-
11	*Salmonella paratyphi*	10	-	-	-
12	*Salmonella Stanley*	-	-	-	-
13	*Salmonella spp*	14	-	-	-
14	*Salmonella typhi*	15	-	-	-
15	*Salmonella typhi*	-	-	-	-
16	*Plesiomona shigelloides*	18	-	-	-
17	*Shigella boydii*	22	15	-	15
18	*Shigella boydii*	14	-	-	-
19	*Shigella dysenteriae*	15	12	-	-
20	*Shigella flexneri*	18	-	-	-
21	*Shigella flexneri*	14	-	-	-
22	*Shigella sonnei*	18	14	15	12
23	*Shigella sonnei*	18	-	-	15
24	*Shigella sonnei*	19	18	12	-
25	*Staphylococcus aureus*	20	-	14	-
26	*Staphylococcus aureus*	18	-	-	-
27	*Staphylococcus aureus*	-	-	-	-
28	*Staphylococcus aureus*	12	12	-	-
29	*Staphylococcus aureus*	14	-	-	-
30	*Staphylococcus aureus*	12	-	-	-
31	*Staphylococcus aureus*	16	-	-	-
32	*Staphylococcus aureus*	14	-	-	-
33	*Vibrio cholerae Inaba*	20	20	-	-
34	*Vibrio cholerae 0139*	16	-	-	-
35	*Vibrio parahaemolyticus*	22	15	17	16

The antibacterial activity revealed by different extracts of *Euphorbia hypericifolia* L. has been shown in Table 3, comparatively as the zone of inhibition in mm affected by different extracts and also on respective test organisms. Four different types of *Euphorbia hypericifolia* L. extracts were tested on the 35 species of bacteria.

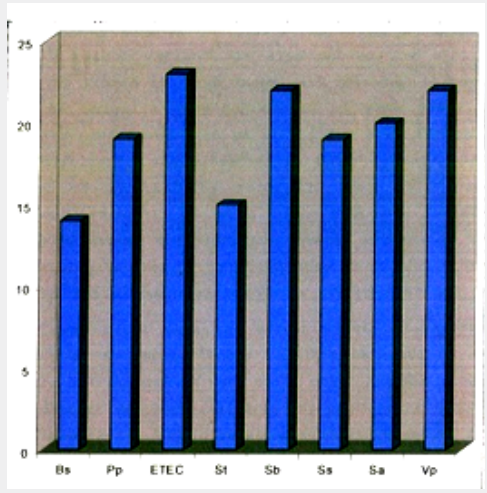
The extracts using 50% ethanol were the most effective and these exhibited antibacterial activity on 29 of the total 35 species tested. Extracts with 95% ethanol showed activity against 9 species, while the extract with ethyl acetate showed antibacterial activity on only 5 species' while that with aqueous solution showed antibacterial activity on only 6 species. It has been noted that the 50% ethanol extract showed the maximum inhibitory zone of 23mm on *E. coli* ETEC and 22mm on *Vibrio parahaemolyticus* (Plate c and d). But it showed minimum antibacterial activity (12mm) on *Proteus morganii* and *Staphylococcus aureus.*

Table 4: *Minimum inhibitory (MIC) concentration of Euphorbia hypericifolia L.(mcg/ml)*

No.	Organisms	50% Ethanol
1	*Bacillus subtilis*	1.0mcg/ml
2	*Klebsella aerogenes*	1.0mcg/ml
3	*Proteus morgani*	1.0mcg/ml
4	*Pseudomonas pyocyanea*	1.0mcg/ml
5	*Escherichia coli ETEC*	0.5meg /ml
6	*Escherichia coli LT*	0.25meg /ml
7	*Escherichia coli EAEC*	1.0mcg/ml
8	*Escherichia coli EHEC*	1.0mcg/ml
9	*Escherichia coli ATCC*	1.0mcg/ml
10	*Escherichia coli EPEC*	1.0meg /ml
11	*Salmonella paratyphi*	1.0mcg/ml
12	*Samonella paralyphi*	1.0mcg/ml
13	*Salmonell spp*	1.0mcg/ml
14	*Salmonell typhi*	1.0mcg/ml
15	*Salmonell typhi*	1.0mcg/ml
16	*Plesiomona shigelloides*	1.0mcg/ml
17	*Shi gel la boydii*	1.0mcg/ml
18	*Shigella boydii*	1.0mcg/ml
19	*Shigella dysenteriae*	0.5mcg/ml
20	*Sh igella flexneri*	1.0mcg/ml
21	*Shigella flexneri*	1.0mcg/ml
22	*Shigella sonnei*	1.0mcg/ml
23	*Shigella sonnei*	0.25meg /ml
24	*Shigell sonnei*	0.25meg /ml
25	*Staphylococcus aureus*	0.25meg /ml
26	*Staphylococcus aureus*	0.5mcg/ml
27	*Staphylococcus aureus*	0.5meg /ml

28	*Staphylococcus aureus*	0.5meg /ml
29	*Staphylococcus aureus*	0.5mcg/ml
30	*Staphylococcus aureus*	0.5meg /ml
31	*Staphylococcus aureus*	0.5meg /ml
32	*Staphylococcus aureus*	0.5meg /ml
33	*Vibrio cholerae Inaba*	0.125mcg/ml
34	*Vibrio cholerae 0139*	1.0mcg/ml
35	*Vibrio parahaemolyticus*	0.0625meg /ml

The 95% ethanol extract of *Euphorbia hypericifolia* also revealed the moderate antibacterial activity of 20mm inhibitory zone on *E. coli* LT as shown in the case of 50% ethanol extract. It also showed the similar effect of *Vibrio* cholerae Inaba (20mm) zone has been shown on *Shigella dysenteriae* (12mm with 95%) and *Staphylococcus aureus* (20mm with 95%). When *Euphorbia hypericifolia* L. was extracted by ethyl acetate, less amount of inhibitory zone (17 mm) on *Vibrio parahaemolyticus* 15mm on *E. coli* ETEC and *Shigella sonnei*) occurred. Moreover, the aqueous extract of the plant also gave the (12 to 17mm) zone on *E.coli, Vibro, Shigella* and *Pseudomonas pyocyanea* (Plate 2. a).



Bs = *Bacillus subuls*
Pp = *Pseudomonas pyocyunea*
ETEC = *Enterotoxigenic Escherichia coli*
St = *Salmonella typht*
Sb = *Shgella boydii*
Ss = *Shigella somei*
Sa = *Staphyl ococcus aureus*
Vp= *Vigrioparahaemolyticus*

Plate 1: *Effect of Euphorbia hypericifolia L. (50% ethanolic extract) on different bacteria.*

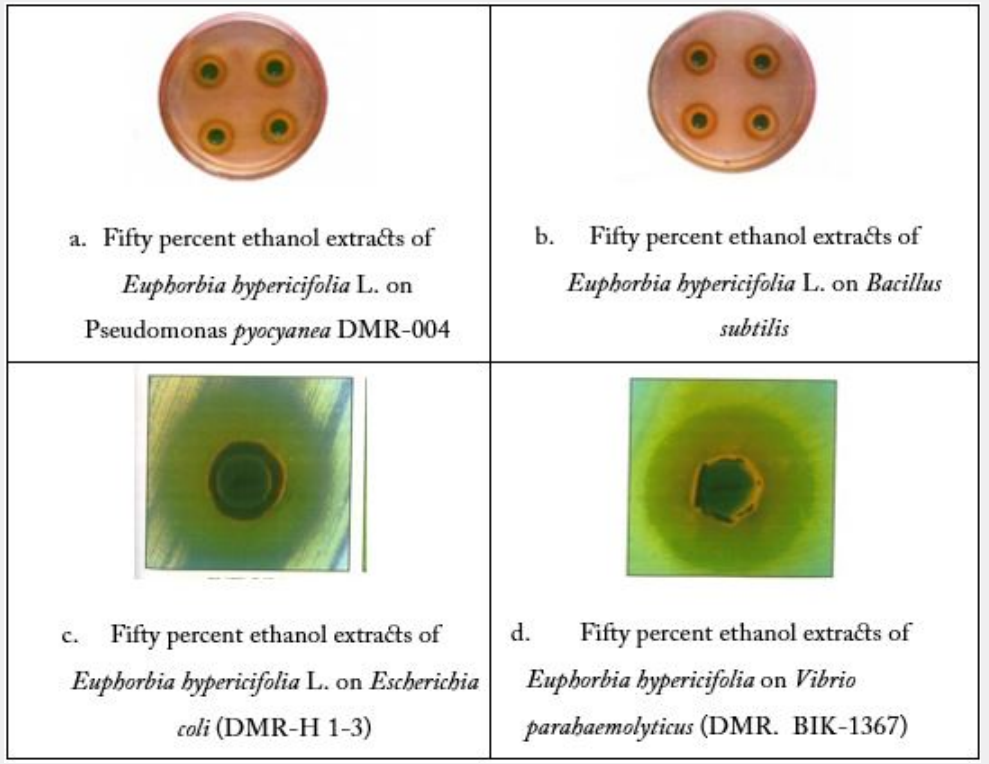


Plate 2: *Effect of Euphorbia hypericifolia L. (50% ethanolic extract) on different bacteria.*

It was clear that the screening of antibaterial activity by *E. hypericifolia* L. plant extracts of 50% ethanol, 95% ethanol as well as of ethyl acetate showed the prominent antibacterial activity on *E. coli* (ETEC), *Shigella sonnei* & *Vibrio parahaemolyticus*. Based on the antibacterial activity results, the 50% extract by ethanol was selected for further investigation.

Determination of Minimum Inhibitory Concentration (MIC) of the Active Extracts

The bacteriostatic and bactericidal activities of the extracts were tested by test tube serial dilution method and determined the turbidity and growth on the nutrient agar [4]. All the tested extracts showed the bactericidal activity which coincide with the MIC values. The minimum inhibitory concentration (MIC) of antibacterial activity 50% ethanolic extracts of *Euphorbia hypericifolia* was shown in Table 4. All the test organisms were the same as the former experiments, MIC ranged from 0.0625mcg/ml to 1mcg/ml with variation on the type of the bacteria used. The least MIC, 0.0625mcg/ml, was obtained. Finally it has been concluded that the extracts by organic solvents revealed the distinct antibacterial activity on a gram negative bacteria such as *E. coli, Salmonella, Shigella* and *Vibrio.*

Vitro Method Using Agar Disc Diffusion Technique

The antibacterial activity of the crude extracts of *Euphrbia hypericifolia* L, was screened by in on 35 bacteria, which include *Bacillus subtilis, Klebsiella aerogenes, Proteus morganii, Pseudomonas pyocyanea, Escher ichia coli, Salmonella, Shigella, Stapyllococcus aureus Vibrio* [1].

EuphorbiahypericifoLia L. extract has a definite antibacterial activity against dysentery, MIC for 50% ethanol extracts against bacteria was determined as 0.0625mcg/ml. The results show that, 95% ethanol extract has been found to have the best inhibition on the *in vitro* growth of Entamoeba histolytica, in monoxenic culture. Fifty percent ethanol extract and aqueous extract also exhibited growth inhibition although to lesser degrees.

Discussion and Conclusion

According to the results, the extracts made by organic solvents revealed the distinct antibacterial activity on gram-negative bacteria such as *E.coli, Salmonella, Shigella, and Vibrio*. It will be much beneficial, if one can produce 50% ethanol extract of *Euphorbia hypericifolia* may be used as a remedy for the treatment of dysenteric patients but we need large randomized control trials to confirm its effect in human model. It was generally found that *Euphorbia hypericifolia* L. had a definite amoebicidal action. It was also confirmed by the recent discovery of its amoebicidal action by Professor J. David Phillipson who is the present Head of Department and Professor of Pharmacognosy in the school of Pharmacy, University of London [6]. According to Ridet and Chartol (1964), Professor Phillipson (1991), there is no toxic action found in *Euphorbia hypericifolia* L. It may therefore be of therapeutic importance on both infants and adults. Moreover, *Euphorbia hypericifolia* L. has long been a popular folk medicine and is also a known and accepted medicinal plant by the people of Myanmar [6,7].

Conflict of Interest

I declare there is no conflict of interest in this research.

Acknowledgment

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