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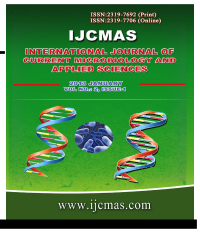

Original Research Article

Antioxidant, antihelmintic and antimicrobial activity of *Euphorbia thymifolia* Linn whole plant

G. Durai Muthumani[1]*, A.Vijay Anand[2] and R.Manikandan[3]

[1]Department of Biochemistry, Kanchi Shri Krishna College of Arts and Science, Kilambi, Kancheepuram -631 551, Tamil Nadu, India.
[2]Department of Biochemistry, MIET Arts and Science College, Gundur, Trichy, Tamil Nadu, India.
[3]Department of Chemistry, SSK Polytechnic College, Poolangulathupatti, Trichy, Tamil Nadu, India.
*Corresponding author: gdbiochm@gmail.com

K e y w o r d s

Euphorbia thymifolia Linn; Biochemical analysis; Mineral content; Antioxidant; Antihelmintic; Antimicrobial activity.

A B S T R A C T

Many plants from *Euphorbia thymifolia* Linn which are available in semi-arid regions like India had shown a variety of pharmacological attributes for different disorders. The present works involved in the evolution or the antioxidant, antihelmintic and antibacterial activity of the plant studied for the proposed activities. Antioxidant compounds such as Beta carotene, vitamin-C, chlorophyll a, b, phenolics and tannins are investigated various concentration of extract were used in the bioassay, which involved paralysis and death time of the worms. The growth of all the bacteria were inhibited though to varying degree thus justifying the use of the herb in traditional medicine in treating enteric infectious, *E.coli*, and *Salmonella typhi* being more susceptible, the antibacterial activity of the plant materials is enhanced under acidic conditions at elevated temperatures.

Introduction

Euphorbia thymifolia Linn in Ayurveda is called as - Chhoti dudhi, Laghu dugdhikaa in Bengal - Dudiya, shweetkeruee, swetkerua, in Ceylon - Cgittirapalavi English - Chicken weed, dwarf spurge, red caustic creeper, in Gujarat - Nahanidudheli In Hindi - Chhoti-dudhi, in Marathi - Ghakdidudhi, Chothadudhi, in Sidha - Ammanptharisi, in Sanskrit - Laghududhika, Raktavindachada, in Spanish - Golondrina Unani - Dudhi khurda Charaka prescribed Dugdhika as an ingredient of vegetable soup for diarrhoea, painful bleeding piles. Hence, the purpose of present work is to evaluate the Anti-inflammatory and Anti-oxidant activities and phytochemical screenings of the ethanolic extract of whole plant of *Euphorbia thymifolia* Linn. Helminthiasis is among the most important animal diseases inflicting heavy production losses. The disease is highly prevalent particularly in third world countries (Dhar *et al*., 1982) due to poor management practices. Chemical control of helminthes coupled with improved management has been the important worm control strategy throughout the world. However, increasing problems of development of resistance in helminths (Geert and Dorny, 1995; Coles,

1997) against antihelmintics have led to the proposal of screening medicinal plants for their antihelmintic activity. The plants are known to provide a rich source of botanical antihelmintics (Satyavati *et al*., 1976; Lewis and Elvin-Lewis, 1977). A number of medicinal plants have been used to treat parasitic infections in man and animals (Nadkarni, 1954; Chopra *et al.,* 1956; Said, 1969; Akhtar *et al*., 2000; Iqbal *et al*., 2004).

In traditional system of medicine the practitioners use various indigenous plants for the treatment of anthelmintic. One such plant drug used by Siddha practitioners is *Enicostemma littorale* commonly called vellarugu is claimed by folklore for various ailments like rheumatism, skin diseases, and constipation. With this view, the *Enicostemma littorale* is studied for its anthelmintic property. There are several reports in the scientific literature describing the antimicrobial properties of crude extracts prepared from plants (Muhammad and Muhammad, 2005; Falodun *et al.,* 2006; El-Mahmood and Amey, 2007) and such reports had attracted the attention of scientists worldwide (Falodun *et al*., 2006; Lai *et al*., 2008). Herbs have been used as sources of food and medicinal purposes for centuries and this knowledge have been passed on from generation to generation (Adedapo *et al.,* 2005). This is particularly evident in the rural areas where infectious diseases are endemic and modern health care facilities are few and far between and where the people nurse their ailments back to health using local herbs.

Most of the pathogens causing enteric infections have developed resistance to the commonly prescribed antibiotics. Bacterial resistance to antibiotics increases mortality, likelihood of hospitalization and the length of stay in the hospital (Winstanley *et al.*, 1997). For most bacteria, there is evidence that increased usage of a particularly antimicrobial correlates with increased levels of bacterial resistance to that agent (Mordi and Erah, 2006). There are several reports in the scientific literature describing the antimicrobial properties of crude extracts prepared from plants (Muhammad and Muhammad, 2005; Falodun *et al.,* 2006; El-Mahmood and Amey, 2007) and such reports had attracted the attention of scientists worldwide (Falodun *et al*., 2006; Lai *et al.,* 2008). Herbs have been used as sources of food and medicinal purposes for centuries and this knowledge have been passed on from generation to generation (Adedapo *et al.,* 2005). This is particularly evident in the rural areas where infectious diseases are endemic and modern health care facilities are few and far between and where the people nurse their ailments back to health using local herbs.

Helminthes are recognized as a major problem to livestock production throughout the tropics (Adejimi and Harrison 1997) parasitic helminthes affect human beings and animals by most diseases caused by helminthes are of a chronic and debilitating in nature. The parasitic gastroenteritis is caused by mixed infections with several species of stomach and intestinal works, which results weakness, loss or appetite, decreased feed efficiency, reduced weight gain and decreased productivity (Gibbs, 1982). Although some of the synthetic drugs are available to control such levels of infectious but due to their high cost and untoward effects, the development of more effective and safe drugs from seasonably less expressive natural sources in our main considerations.

There is great need to evaluate the local herbs for mineral and nutrient composition, so as to determine the potential of indigenous source of medicines (Rahila *et al.,* 1994). *Euphorbia thymifolia* Linn. (Euphorbiaceae) is traditionally used as blood purifier, cough, antiviral in bacterial asthma and paronychia

(Manickam and Rajappan,1998). Water extract of this plants have antiviral activity (Anonymous, 1952; Lin *et al.,* 2002). *E.thymifolia* Linn**.** are numerous reservoirs of many minerals, antioxidants and nutritive properties. Their mineral antioxidant and nutritional compounds are not determined despite their impotence in traditional medicine (Prabha and Singh, 2005). Over the years, man has acquired extensive knowledge regarding the utilization of plants.

Antioxidants are integral part of the nutraceutical market. Last few years of research has confirmed that many of the common diseases and ailments of the 21st century(Cardiovascular diseases, diabetes, cataracts, blood pressure, infertility, respiratory infections, rheumatoid arthritis, Alzhemer's disease, several types of cancer, mental illness, including tumour promotions and AIDS) are associated with tissue deficiency and low dietary level of compound called "antioxidant". Vitamin c is known to be a potential antioxidant and it is essential for functioning of the central nervous system and help in fighting infectious diseases. Vitamin a and carotenoids can both accept and donate electrons and carotenoids can also quench singlet Oxygen. Flavonoids are the major class of phenolic and have been recognized for having a potential role in the prevention of several forms of cancer and cardiovascular diseases. The green leafy vegetables are one of the source of nutrients for growth in man and animals. In developing countries there is need for a constant search of new food values. Because of its wide usage and easy availability, this study was undertaken to investigate the phytochemical properties, antioxidant, antihelminthic and antibacterial activities of the whole plant. *E. thymifolia* Linn effect of pH and temperature on the efficacy of the solvent extracts were also investigated.

Materials and Methods

Plant collection and phytochemical screening

The whole plant of *E. thymifolia* Linn used for the investigation was collected from the area of Ariyaperumpakkam, Kancheepuram, Tamil Nadu (State), India.

Preparation of Ethanolic Extract of *Euphorbia thymifolia* Linn

The whole plant of *E.thymifolia* Linn was collected and coarsely powdered. The powder was successively extracted with ethanol using soxhlet extractor. The ethanolic extract of *Euphorbia thymifolia* was dried under reduced pressure using a rotary flash evaporator and was kept under the refrigeration. The percentage yield was 6%. The ethanolic extract thus obtained was used for the preliminary phytochemical screening and pharmacological studies.

Biochemical analysis

The moisture content was estimated by dried in electrical oven at 80 degree for 24 hour 60 mesh size. The fine leaves powders so obtained were used for further biochemical and mineral analysis (three replication of each parameter). The carotenoids in plant sample were extracted, as described by Ranganna (1976) and Withaam *et al*. (1971) methods. The chlorophyll content in dry leaves powder was estimated by method of Singleton *et al* (1999) with modification. Dry leaves powder (0.5g) was extracted with 10 time volume of 80% ethanol. The homogenate was centrifuged at 10,000 rpm for 20 min and the supernatant was collected. The residue was extracted three times with 80% ethanol, centrifuged and supernatant was collected. The supernatant was evaporated to dryness. The residue was dissolved in 5ml double distilled water and 1.0 mL aliquots were added to 0.5ml Folin-Ciocalteau reagent,

followed by addition of 2.0 mL of 20% sodium carbonate solution and the absorbance measured at 650 nm. Tannins content was estimated as described by method of Schanderi (1970). Ascorbic acid content was estimated by method of Ranganna (1976) with modification. Dry leaves powder (2.0g) was extracted with 4% oxalic acid and made up to 100mL and centrifuged at 10,000 rpm for a 10 min. Five millilitre supernatant liquid was transferred in a conical flask, followed by addition of 10 mL 4% oxalic acid and titrated against standard dye solution (2.6 dichlorophenol-indophenol) to a pink end point. The procedure was repeated with a blank solution omitting the sample. Total carbohydrate content in plant leaves was estimated by Du Bois *et al.* (1956), Starch by Hodge and Hofreiter (1962). Total nitrogen was estimated by Micro-Kjeldahl method, according to AOAC (1985). Crude protein was calculated as Kjeldahl NX6.25(based on assumption that nitrogen constitutes 16.0% of a protein). The content of crude fat was estimated by AOAC (1970). Amylose content in plant leave was estimated, as described method of Mc Cready *et al*. (1950).Cellulose content was estimated as described by method Updegraff (1969). Crude fiber content was estimated as described by methods of Maynard (1970).

Mineral analysis

Ash content was estimated by AOAC (1985) and Mineral content in plant was estimated by wet digestion method. A 1.0g plant material was first digested with cibc. HNO_3 (5mL each), followed by application of 15mL of tri-acid mixture (HNO_3,$HCLO_4$ and H_2SO_4,10:4:1,v/v) heated at 200°Cand reduces to 1 mL. The residue after digestion was dissolved in double distilled water, filtered and diluted to 100mL. This solution was used for the estimation of minerals. Macro minerals *viz*, Fe, Cu, Mn, Zn and Co were estimated by Atomic Absorption Spectrophotometer, model 4129, Electronic corporation of India Ltd. Phosphorous and sulphur content was estimated by method of Allen (1977).

In-Vitro Antioxidant Studies

Nitric Oxide scavenging activity

Nitric oxide scavenging activity was measured by using UV-Visible spectrophotometer. Sodium nitroprusside (5mM) in phosphate buffer was mixed with different concentrations of ethanolic extract *E.thyimifolia* Linn (25-800 μg/ml), dissolved in normal saline and incubated at 25°C for 30 min. Control without test compound but with equivalent amount of sodium nitroprusside was taken. After 30min 1.5 ml of the incubation solution was removed and diluted with 1.5 ml of griess reagent (1% Sulphanilamide, 2% Phosphoric acid, and 0.1% Naphtyl ethylenediamine dihydrochloride). The adsorbents of the chromophore formed during diazotization of the nitrate with sulphanilamide and subsequent coupling with Naphthyl ethylenediamine dihydrochloride was measured at 546 nm. Vitamin-E was used as reference standard.

Antihelmintic activity

The antihelmintic activity was performed according to the method (Ghosh *et al*, 2005).On adult Indian earth worm *Pheretima pothuma* (earth worm) as it has anatomical and physiological resemblance with the intestinal round worm parasites of human beings. *Pheretima pothuma* was placed in petridish containing four different concentrations (25, 50,100,200mg) each of *Enicostemma* (ethanol, and water extract) solutions. Each petridish was placed with 6 worms and observed for paralysis (or) death. The mean time for paralysis was noted

when no movement of any sort could be observed, except when the worm was shaken vigorously; the time death of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli. In the same manner albendazole was included as reference compound. The test results were compared with Reference compound Albendazole (15mg/ml) treated samples.

Preparation of the extracts for antibacterial activity

100 g of the powdered sample (whole plant) was soaked in 100 ml of solvent contained in a 500 ml sterile conical flask and covered with cotton wool. It was then plugged and wrapped with aluminium foil and shaken vigorously. The mixture was left to stand over night (24 h) in a shaking water bath maintained at 40°C. The mixture was then filtered using a clean muslin cloth and then Whatman No. 1 filter paper. The filtrate was then evaporated to dryness using a rotary evaporator attached to a vacuum pump (Model type 349/2, Corning Limited). The percentage yield of the crude extract was determined with solvent ethanol. The percentage extract yield was estimated as dry weight/dry material weight x 100 (Parekh and Chanda, 2007). For the preparation of dilutions of ethnaol extracts for antibacterial assay, the extracts was reconstituted by dissolving in the respective extracting solvents and further diluted to obtain 400, 200, 100, 50, 25, 12.5, 6.25, 3.085 and 1.03 mg/ml. The reconstituted extracts were maintained at a temperature between 2 - 8°C.

Microorganisms used

The bacteria of clinical isolates of *E. coli*, *Proteus mirabilis*, *Shigella dysentriae*, *Salmonella typhi* and *Klebsiella pneumoniae* were used for antimicrobial study.

Determination of the antimicrobial activity

The method described by Emeruwa (1982) was used. Briefly, 1.0 ml of 18 h culture of bacteria adjusted to 1.0 x 10 8 cfu/ml was spread into a sterile plate so as to achieve a confluent growth. 3 petri dishes containing a particular bacteria was used. Then 19.0 ml of Mueller Hinton agar at 45°C was added to each plate and the plates were rocked for even spread and proper mixing of bacteria and agar.

The content of the plates were allowed to solidify and wells approximately 6 mm in diameter and 2.5 mm deep were bored on the surfaces of the agar medium using a sterile cork borer. Then 0.5 ml of the reconstituted extract at a concentration of 100 mg/ml was pipetted in to one of the holes. 0.5 ml of pure solvent was pippeted into another hole as negative control while an aqueous solution of 12.5 ug amoxicillin was used as positive control. The plates were allowed to stand for 1 h for prediffusion of the extract to occur and then incubated at 37°C for 24 h and the zones of inhibition were measured to the nearest mm. The mean of triplicate results were taken.

Determination of MIC and MBC

Determination of the minimum inhibitory concentration (MIC) was carried out using the Broth dilution method (Sahm and Washington, 1990; Adesokan *et al*., 2007; Oyeleke *et al*., 2008). Briefly, 1.0 ml of the reconstituted extract solution at a concentration of 200 mg/ml was added to another test tube containing 1 ml of sterile broth so as to obtain a concentration of 100 mg/ml. 1 ml of this dilution was transferred to another test tube till the 7th test tube was reached.

The 8th test tube did not contain any extract, but a solution of pure solvent and served as negative control. Then 1 ml of an 18 h old culture of each of the bacteria earlier adjusted at 108cfu/1ml was put into each tube and thoroughly mixed on a votex mixer. The tubes were incubated at 37ºC for 24 h and observed for growth in form of turbidity. The test tube with the lowest dilution with no detectable growth by visual inspection was considered the MIC.

The MBC values were determined by removing 0.10 ml of bacterial suspension from the MIC tubes that did not show any growth and subcultured into Mueller Hinton agar plates and incubated at 37°C for 24 h. After incubation, the concentration at which no visible growth was seen was recorded as the MBC.

Effects of pH on solvent extract of *E.thymifolia* Linn

This was carried out as previously described (El-Mahmood *et al.,* 2008). Briefly, the extracts were reconstituted in to 3 separate test tubes each containing 100 mg/ml of extract in 4 ml test tubes. Then 1 ml of an 18 h old culture of each of the bacteria earlier adjusted at 108 cfu/1ml was put into each tube and thoroughly mixed on a votex mixer. The first tube was treated with 1N hydrochloric acid by adding it drop wise until a pH of 2 was obtained.

The second tube was treated with 1M sodium hydroxide by adding it drop wise until a pH of 10 was reached. The test tubes were left to stand for 1 h and then neutralized by acid or alkali treatment as the case might be. The third test tube was not treated and served as control. The test tubes were incubated at 37°C for 24 h. Antibacterial activity was determined as previously described.

Effect of temperature on solvent extract of *E.thymifolia* Linn

The effects of temperature on the efficacy of the crude extracts was determined by reconstituting the powdered extracts to obtain a concentration of 100 mg/ml in 3 separate test tubes of 4 ml each. Then 1 ml of an 18 h old culture of each of the bacteria earlier adjusted at 108 cfu/1ml was put into each tube and thoroughly mixed on a votex mixer. The first test tube was treated at a temperature of 10 °C in a refrigerator for 1 h and the second test tube was treated at a temperature of 100°C in a water bath, also for 1 h, after which both test tubes were removed and left to acclimatize at room temperature. The third test tube was not subjected to either cold or heat treatment and served as control.

Results and Discussion

Phytochemical Screening of Ethanolic Extract of whole plant of *E.thymifolia* Linn

The results of preliminary phytochemical screening of the ethanolic extract of whole plant *E.thymifolia* Linn was shown the presence of alkaloids, carbohydrates, phenols, sterols, terpenes and flavonoids by doing various confirmatory tests for each type of chemical constituents and shown in Table-1.

Antioxidant activity of *E.thymifolia* Linn

Nitric Oxide scavenging activity *E.thymifolia* Linn showed promising free radical scavenging effect against nitric oxide induced release of free radicals in a concentration dependent manner. The IC_{50} values of ethanolic extract of *E.thymifolia* Linn was found to be 638.36µg/ml (r = 0.932) and 645µg/ml(r = 0.921), respectively. The IC_{50}value of vitamin E was 142.2µg/ml (r =

0.909). Antioxidant content in whole plants of *Euphorbia* is presented in table 2. Beta-carotene of plants was found 307.40 mg/100g. The content of Vitamin C of whole plants was found 88.48 mg/100g on dry weight basis. The content of chlorophyll-a and chlorophyll-b in aerial parts of plants were found 115.37 and 72.98 mg/100g on dry weight basis. The content of phenolic and tannins in plant was found 336.73 and 2465.74 mg/100g.

Biochemical analysis *of E.thymifolia* Linn

Crude lipids, proteins and total carbohydrates content in of the plants were found to be 4.63, 13.42 and 11.99 g/100g respectively. Starch, amylose and amylopectin content of *Euphorbia* sp. were found 22.31, 1.39 and 20.93g respectively. Cellulose, crude fibre and moister content were found 4.35, 24.34 and 78.60 g/100 g respectively. Ash and acid soluble and insoluble ash energy content caloric value of the plant represented in table.3.

Mineral analysis of *E.thymifolia* Linn

Minerals are called spark plugs of life because they are required to activate hundreds of enzymes reactions within the body. Life is dependent upon the body's ability to maintain balances between the minerals (Watts, 1997). The contents of sodium, potassium, calcium and lithium of whole plants are 75.40, 4786.48, 242.46 and 46.64 mg/100g respectively. The contents of N, P, S, iron, copper, Manganse, zinc, and cobalt were represented in table.4. This study was well accepted with previous study by Watts, (1997), Karade *et al*., (2004), Barasi and Mottram, (1987).

Table.1 Phytochemical screening of Ethanolic Extract of whole plant of *E.thymifolia* Linn

Chemical Tests	Inference	Constituents
a. Mayer's test b. Dragendorff's test c. Hagner's test	Positive Positive Positive	Alkaloids Present
a. Molisch's test b. Fehling's test c. Benedict's test d. Barfoed's test	Positive Positive Positive Positive	Carbohydrates Present
a. Libermann-Burchard test b. 5% KOH test	Positive Positive	Steroids Present
a. Biuret test b. Millon's test	Negative Negative	Proteins Absent
a. 10% Lead acetate b. 10% NaCl, c. Aq. Bromine solution tests	Positive Positive Positive	Tannins Present
a. Ferric Chloride b. 10% Sodium Chloride tests	Positive Positive	Phenols Present
a. Conc. Sulpuric acid b. Magnesium turnings test c. Shinoda test d. Lead acetate test e. Sodium hydroxide test	Positive Positive Positive Positive Positive	Flavonoids Present
a. Swelling test	Negative	Gums & Mucilage Absent
1. Baljet test 2. Borntrager's test 3. Modifies borntrager's test	Positive Positive Positive	Glycosides present
a. Froth test	Positive	Saponins Absent
a. Tin + Thionyl Chloride test	Positive	Terpenes Present
a. Spot test	Negative	fixed oils Absent

Table.2 Antioxidant phytochemical composition investigated in whole plant *E.thymifolia*

Antioxidants	Composition (mg/100g)*
Beta-Carotene	307.40
Vitamin C	88.48
Chlorophyll a	115.37
Chlorophyll b	72.98
Phenolics	336.73
Tannins	2465.74

*All values are Mean of triplicate determination expressed on dry weight basis.

Table.3 Nutrients composition investigated in whole plants of *E.thymifolia*

Biochemical parameters	Composition (mg/100g)*
Moisture	78.60
Crude protein	13.42
Crude fat	4.63
Total carbohydrate	11.99
Starch	22.31
Amylose	1.39
Amylopectin	20.93
Cellulose	4.35
Crude fibre	24.34
Ash	10.29
Acid soluble ash	7.07
Acid insoluble ash	3.24
Calorific value	143.31

*All values are Mean of triplicate determination expressed on dry weight basis

In vitro antihelmintic activity

The solvent extracts samples, which were used to evaluate anthelmintic activity, showed variable times at different concentrations and the mean time values were calculated for each parameter. The extracts of ethanol showed the significant anthelmintic effect causing death of the worm at all the concentrations but the time of death was different in each case. However, when observed the response of worms in case of paralysis, there was significant variation among the results produced by the different extracts at different concentrations like 10, 50, 100, mg/ml. The ethanol extract showed more significant effect on paralyzing the worms, in terms of paralysis time, at every concentration compared to that of water and ethanol extracts. Similar observations were made in the anthelmintic activity as well (Table.5). Tannins, the secondary metabolite, occur in several plants have been reported to show antihelmintic property by several investigators (Athnasiadou *et al.*,; 2001; Waller *et al*., 1997). Tannins, the polyphenolic compounds, are shown to interfere with energy generation in helminthic parasites by uncoupling oxidative phosphorylation or, binds to the glycoprotein on the cuticle of parasite (Thompson and Geary, 1995), and cause death. Coming to the chemistry of nematode surface, it is a collagen rich extracellular matrix (ECM) providing protective cuticle that forms exoskeleton, and is critical for viability, the collagen is a class of proteins that are modified by a range co-and post – translational modification prior to assembly into higher order complexes (or) ECMS (Page and Winter, 2003).

Table.4 Minerals composition investigated in whole plants of *E.thymifolia*

Minerals	Composition (mg/100g)
Sodium	75.40
Potassium	4786.48
Calcium	242.46
Lithium	46.64
Nitrogen	2151.85
Phosphorus	226.81
Sulphur	325.24
Iron	121.04
Copper	3.45
manganese	9.28
Zinc	6.47
Cobalt	0.00

Antibacterial activity of *E.thymifolia* Linn extract

Antibacterial activity of ethanol extracts of *E.thymifolia* were evaluated by measuring the diameters of zones of growth inhibition on some members of the Enterobacteriaceae and the results are presented as shown in Table 6. All the test organisms were susceptible to *E.thymifolia* extracts though to varying degrees. Karou *et al.* (2006) reported that the susceptibility of bacteria to plant extracts, on the basis of inhibition zone diameters varied according to strains and species, similar to the data obtained in this study. The highest zone of growth inhibition was shown by ethanol extract against *E. coli* (18.0 mm), *K. pneumonia* (18 mm), P. mirabilis (19 mm), *S. dysentriae* (26 mm) and *S. typhi* (16 mm). Aqueous extract gave inhibitory zones of 13 mm for *E. coli*, 16 mm for *P. mirabilis*, 10 mm for *S. typhi*, 10 mm *S. dysentriae* and 20 mm for *K. pneumoniae*. All the bacteria used in this study are gram negative bacteria which are known to be resistant to the action of most antimicrobial agents including plant based extracts and these have been reported by several scholars (Geyid, 2002; Kambezi and Afolayan, 2008). Gram negative bacteria have an outer phospholipids membrane with the structural lipopolysaccharide components, which make their cell wall impermeable to antimicrobial agents (Nikaido and Vaara, 1985). However, Afolayan *et al*. (2002) have reported the inactivity of the shoots of *Helichrysum* aureonitens against some *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *S. marcescens.* The water extracts are more effective than methanol and hexane extracts. The large zone sizes produced by the plant extract against the test bacteria, especially the ethanol extracts is an indication of the potency of the bioactive components of the plant against all the test bacteria. Amoxicillin which served as positive control and at lower concentrations produced larger zone diameters for *E. coli, K. pneumoniae, P. aeruginosa, S. dysentriae, P. mirabilis* and *S. typhi* than the ethanolic extracts.

Table.5 In vitro antihelmintic activity of various extracts *E.thymifolia* Linn Group

Test substances	Conc.	Time taken for paralysis	Death
Control	-	-	-
Ethanol extract	10	26.5	65.0
	50	17.0	45.5
	100	9.66	30.5
Aqueous extract	10	29.17	67.83
	50	19.67	50.67
	100	08.66	33.33
Pipeerazine citrate	10	18.83	60.33

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of crude extracts of *Euphorbia thymifolia*

Aqueous extract showed minimum inhibitory concentration (MIC) for *E. coli* (50 mg/ml), *P. mirabilis* (25 mg/ml), *S. typhi* (50 mg/ml), *S. dysentriae* (25 mg/ml) and
for *K. pneumoniae* (50 mg/ml) as detailed in Table 7. The MIC values produced by the ethanol extract for *E. coli* and *S. typhi* were 100 mg/ml, while those for *S. dysenteriae, P. mirabilis* and *K. pneumobiae* were 50 mg/ml respectively. The MIC values for hexane followed similar pattern. The aqueous extract gave an MBC of 100 mg/ml for *E coli and S. typhi* respectively, while for *S. dysentriae, K. pneumoniae, P. mirabilis,* the MBC values were 50 mg/ml, respectively. In the case of ethanolic extract, the MBC recorded was 100 mg/ml for *E. coli, P. mirabilis, K. pneumoniae* and *S. typhi* respectively and for *S. dysentriae* the MBC

value was 50 mg/ml. The effects of the solvent extracts correlate with reports that microorganisms varied widely in their degree of susceptibility (Emeruwa, 1982; Banso and Mann, 2006). The results obtained here are similar to those presented by Adesokan *et al.* (2007). Hugo and Russell, (1984) have reported that the MBC values can either be the same or higher than the MIC values. In this study, the MIC values were either the same or slightly lower than the MBC values, similar to the results of Karou *et al*. (2006).

Effects of pH

The effects of pH on the activity of the extracts are given in table 8. For aqueous extracts, the zone diameters are: 28 mm at pH 2 and 24 mm at pH 10 for *E. coli*, 20 mm at pH 2 and 18 mm at pH 10 for *P. mirabilis,* 27 mm at pH 2 and 23 mm at pH 10 for *S. typhi*, 21 mm at pH 2 and 17 mm at pH 10 for *S. dysentriae* and 22 mm at pH2 and 20 mm at pH 10 for *K. pneumoniae*. A similar growth inhibition was exhibited by a ethanol extract: 30 mm pH 2 and 26 mm at pH 10 for *E. coli*, 22 mm at pH 2 and 18 mm at pH 10 for *P. mirabilis*, 25 mm at pH 2 and 23 mm at pH10 for *S. typhi*, 23 mm at pH 2 and 21 mm at pH 10 for *S. dysentriae* and 25 mm at pH 2 and 23 mm at pH 10 for *K. pneumoniae*.. For all the extracts, activity was more under acidic than alkaline conditions. Similar observations were made by Doughari *et al.* (2008) and El-Mahmood *et al.* (2008). Acid stability is an important property of drugs, because it means that the plant components can be formulated to be taken orally and will not be inactivated under the acidic conditions of the stomach and the gastrointestinal tract.

Effects of temperature

The temperature was adjusted between 10 and 100°C for all extraction solvents. For aqueous extract, *E. coli* was inhibited by a zone diameter of 17 mm at 100°C and 13 mm at 10°C ,for *P. mirabilis*, the zone diameter were 15 mm at 100 °C and
13 mm at 10 °C, *S. typhi* gave a zone of growth inhibition diameter of 15 mm at 100°C and 12 mm at 10 °C, *S. dysentriae* gave a zone of growth inhibition diameter of 14 mm at 100°C and 11 mm at 10°C and *K. pneumonia* inhibited the growth of the bacteria with zone diameters of 16 mm at 100°C and 13 mm at 10°C. For ethanolic extract, *E. coli* gave a zone diameter of 18 mm at 100 °C and 14 mm at 10°C, *P. mirabilis* gave a zone diameter of 15 mm at 100°C and 13 mm, *S. typhi* gave a zone diameter of 18 mm at 100°C and 15 mm at 10°C, the diameter of zone of growth inhibition for *S. dysentriae* was 14 mm at 100°C and 12 mm at 10°C while *K. pneumonia* gave a zone diameter of 17 mm at 100°C and 13 mm at 10°C. As the temperature was increased, the antibacterial activity also increased, similar to the data presented by Doughari *et al*. (2008) and El-Mahmood *et al*. (2008). The traditional practitioners usually boil the plants before dispensing out to patients. The results obtained in this study support the methods used by the traditional healers. It is evident from the results that water extract has some significantly high antibacterial activity, suggesting that the active principles are more soluble in water and that water is the appropriate solvent for the extraction of the bioactive principles present in *E. thymifolia*, similar to the reports of Falodun *et al*. (2006) and El-Mahmood and Amey (2007), but contrary to that of Banso and Mann (2006). This is a clear indication that the solvent system plays a significant role in the solubility of the active principles in the plant and influences the antibacterial activities. The efficacy of the extracts, probably due to the identified secondary metabolites, further confirm its use as an antibacterial agent in folkloric medicine and may thus be useful in

Tables. 6 Antibacterial activity of *Euphorbia thymifolia* Linn

Organisms	Ethanol extract	Aqueous extract
E.coli	19.0	15.0
K.pneumoniae	20.0	20.0
S.typhi	16.0	10.0
P.mirabilis	18.0	14.0
S.dysenteriae	26.0	10.0

Table. 7 The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of crude extracts of *Euphorbia thymifolia*

Organisms	Aqueous extract		Ethanol extract	
	MIC	MBC	MIC	MBC
E.coli	50	100	100	100
K.pneumoniae	25	50	50	100
S.typhi	50	100	100	100
P.mirabilis	25	50	50	100
S.dysenteriae	25	50	50	50

Table. 8 Effect of pH on the antibacterial activity of *Euphorbia thymifolia*

Organisms	Aqueous extract		Ethanol extract	
	pH2	pH10	pH2	pH10
E.coli	28	24	30	26
K.pneumoniae	22	20	25	23
S.typhi	27	23	25	23
P.mirabilis	20	18	22	18
S.dysenteriae	21	17	23	21

Table. 9 Effect of temperature on the antimicrobial activity of *Euphorbia thymifolia*

Organisms	Ethanol extract		Aqueous extract	
	100ºC	10ºC	100ºC	10ºC
E.coli	18.0	14.0	17.0	13.0
K.pneumoniae	17.0	13.0	16.0	14.0
S.typhi	18.0	15.0	15.0	12.0
P.mirabilis	15.0	13.0	15.0	13.0
S.dysenteriae	14.0	12.0	14.0	11.0

the treatment of enteric infections. The plant can be used to source for oral antibacterial drugs that can treat infections caused by phytochemical and Pharmacological studies will be needed to isolate the active constituents and evaluate the antimicrobial activities against a wide range of microbial pathogens.

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