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REGULAR ARTICLE

ETHNO-MEDICINAL, PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES OF *EUPHORBIA TIRUCALLI* L.

Bhuvaneshwar Upadhyay*, K.P. Singh and Ashwani Kumar

Biotechnology lab, Department of Botany & P. G. School of Biotechnology,
University of Rajasthan, Jaipur, India

SUMMARY

Present study exposed various claims about the medicinal properties of *Euphorbia tirucalli* L., used by the indigenous people of Rajasthan to cure rheumatism, Skin disorders, Cough and other ailments. This plant was assessed for ethnopharmacological screenings, phytochemical analysis and antimicrobial screenings which also include anti-HIV activity, so as to validate the efficacy of indigenous herbal medicine. In the present study antimicrobial activity of the crude alcoholic extracts of leaf and stem of *E. tirucalli* against the known enteric pathogens was carried out. Anti-HIV screening activity was carried out using HIV Protease colorimetric Assay. Low MIC exhibited by the extract against *S. aureus* is of great significance in the healthcare delivery system, since it could be used as an alternative to orthodox antibiotics in the treatment of infections caused by these microbes, especially as they frequently developing resistance to known antibiotics.

Key words: Alkaloids, Triterpenes, Antimicrobial activity, anti-Carcinogenic, Anti-HIV

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*Corresponding Author, Email: bhuvan.com@gmail.com

1. Introduction

Current research on natural molecules and products primarily focuses on plants, they can be sourced, and selected more easily based on their ethno-medicinal use (Verpoorte *et al.*, 2005). Plant derived medicines have been part of traditional health care system in most parts of the world for thousands of years and nowadays there is increasing interest in plants as sources of agents to fight microbial diseases (Natarajan *et al.*, 2005).

The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. Plants produce secondary metabolites as defenses against animals, parasites, bacteria, and viruses, and so rely on these chemical and other deterrents for their survival. These secondary metabolites constitute the medicinal value of a drug plant, which produces a definite physiological action on human body (Sharma *et al.*, 2007).

Many studies focus on determining the antimicrobial activity of plant extracts, found in folk medicine (Ngwendson *et al.*, 2003), essential oils (Alma *et al.*, 2003; Maria *et al.*, 2008) or isolated compounds such as alkaloids (Klausmeyer *et al.*, 2004; Vanessa *et al.*, 2008), sesquiterpene lactones (Lin *et al.*, 2003), triterpenes (Katerere *et al.*, 2003) or naphtoquinones (Machado *et al.*, 2003), flavonoids (Sohn *et al.*, 2004), diterpenes (Siegfried *et al.*, 2006), etc. Some of these compounds were isolated or obtained by bioactivity-guided isolation after previously detected antimicrobial activity on the part of the plant.

Euphorbia tirucalli L. (Family, Euphorbiacae) a succulent cactus-like plant growing to a height of about 10 m, was introduced from Africa as a garden plant. *E. tirucalli* grows in arid zones as well as zones that are more mesophytic, the species makes a good living fence post. The plant grows well in dry regions or land that is not suitable for growing food. *E. tirucalli* is called *petroleum plant* because it produces a hydrocarbon substance very much like gasoline. Whole plant harvesting is worthwhile from energy point-of-view with rubber, petroleum, and alcohol as energy products and resins, which may find use in the linoleum, oilskin, and leather industries. The charcoal derived there from can be used in gunpowder.

Many pharmacological activities of *E. tirucalli* have been documented by many workers as molluscicidal activity (Jurberg *et al.*, 1985; Tiwari *et al.*, 2003), antibacterial activity (Lirio *et al.*, 1998), antiherpetic activity (Betancur-Galvis *et al.*, 2002) and anti-mutagenic (Rezende *et al.*, 2004). Latex also shows co-carcinogenic (Gscwhenot and Hecker, 1969) and anti-carcinogenic activities (Hecker, 1968). The inhibition of the ascitic tumor in mice has also been reported by Valadares *et al.* (2006). In the northeast region of Brazil, the latex of *E. tirucalli* is used; as an antimicrobial agent; a laxative agent; to control intestinal parasites; to treat asthma, cough, earache, rheumatism, verrucae, cancer, chancre, epithelioma, sarcoma, skin tumors and as a folk remedy against syphilis (Correia, 1994; Betancur-Galvis *et al.*, 2002).

Exposure to *E. tirucalli* has been suggested as an important environmental risk factor for African Burkitt's lymphoma (Van den Bosch *et al.*, 1993; Imai *et al.*, 1994; MacNeil *et al.*, 2003). In *E. tirucalli* 4-deoxyphorbol ester, has been clinically documented to enhance Epstein-Barr virus (EBV) infection, causing damage to immune cell's DNA and induce rearrangement in the chromosomes, particularly in chromosome 8, which causes a suppression of the immune system (Aya *et al.*, 1991; Jurberg *et al.*, 1985; Almeida, 1993; Costa, 2002; Tiwari *et al.*, 2003).

The stem contains alcohol eufol, α-euforbol and taraxasterol, tirucallol (Costa, 2002; Almeida, 1993), hentriacontene, hentriacontanol, the antitumor steroid β-sitosterol, taraxerin, 3,3′-di-O-methylellagic acid, ellagic acid, and a glycoside fraction which hydrolyses to give kampferol and glucose. The whole plant contains 7.4% citric acid with some malonic and some bernstein (succinic) acids (List and Horhammer, 1969). The latex of *Euphorbia tirucalli* contains as irritant constituents ingenane- and tigliane-type diterpene esters derived from the parent alcohols ingenol and phorbol (Furstenberger and Hecker, 1986). The main irritant constituents are isomeric 12,13-acetates, acylates of phorbol as well as 3-acylates of ingenol (Imai *et al.*, 1994). In the acyl moiety of phorbol esters investigated in detail, an increasing number of C-atoms or an increasing number of double bonds at a fixed number of C-atoms leads to an increase of irritant activity.

As compared to their saturated analogs, corresponding unsaturated phorbol esters exhibit similar irritant activities (Duke, 1983). On the other hand, by an increasing number of conjugated double bonds in the acyl moieties of phorbol esters, the promoting activity is decreased, thus indicating that irritant activity is a necessary, but insufficient, requirement for promoting activity of phorbol esters (Furstenberger and Hecker, 1977). The latex contains 53.8-79.9% water and water solubles and 2.8-3.8% caoutchouc. Fresh latex contains a terpenic alcohol, isoeuphorol ($C_{30}H_{50}O$), Dried latex contains no isoeuphorol but a ketone euphorone ($C_{30}H_{48}O$) (Uzabakiliho *et al.*, 1987). Resin, however, is the principal constituent (75.8-82.1%) of the dried latex. The stem contains hentriacontene, hentriacontanol, the antitumor steroid 4-deoxy-phorbol ester, beta-sitosterol, caoutchouc, casuariin, corilagin, cycloeuphordenol, cyclotirucanenol, ellagic acids, euphorbins, euphol, euphorone, euphorcinol, gallic acids and glucosides (Khan and Malik, 1990). Therefore the present study has been undertaken to investigate the antimicrobial activity of leaf extract of *Euphorbia tirucalli* by disc diffusion method.

2. Materials and Methods

Extraction of the plants

The leaves of *E. tirucalli* were collected from regional areas of Jaipur city, during post monsoon period and were authenticated by botanists at Dept. of Botany, University of Rajasthan, Jaipur, India and a specimen sample is kept in our institution (herbarium voucher numbers RUBL 20279). Shade dried coarsely powdered leaves (44 g) and stem (36 g) of *E. tirucalli* were subjected to successive extraction with methanol (54-55.5°C) for 24-36 hr using a soxhlet extractor separately. These crude extracts were concentrated using vacuum evaporator. The extract yield was 2.6g (5.9%) and 16 g (4.44%) respectively. Percent yield was calculated by using following formula. One gram of the dried filtrate was reconstituted with 10 ml of 100% dimethylsulfoxide (DMSO).

Paper disks (diameter 6mm) were then impregnated with 25µl, 50µl, and 75µl of the final extract, which is equivalent of 2.5, 5, and 7.5mg/ml of dried plant material. Filter paper discs (Whatman No. 1) of 5mm diameter were loaded with 1 ml of crude extracts. Once the DMSO had evaporated, the disks were placed in a refrigerator and stored in darkness for the duration of the assays. 0.01ml of one of the 24 h broth cultures culture (10^5 bacteria per ml) were spread on sterilized nutrient agar media and impregnated discs were placed on it and incubated for 24 h at 37°C.

$$\%\ \text{Yield} = \frac{\text{Weight of extract}}{\text{Dried weight of Sample}} \text{ X } 100$$

Preparation of micro-organism culture

In vitro antimicrobial activity of the different extracts of *E. tirucalli* was studied by disc diffusion method using different concentrations on different microbial strains such as *Escherichia coli* (ATCC 25922 and Clinical isolate), *Proteus vulgaris* (ATCC 13315), *Salmonella enteritidis* (clinical isolate), *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538P and clinical isolate), *Pseudomonas aeruginosa* (ATCC 9027 and clinical isolate), *Klebsiella pneumoniae* (ATCC 13883), *Candida albicans* (ATCC10231 and clinical isolate), *C. tropicalis* (clinical isolate), *Aspergilus niger, A. fumigatus, A.flavus* and *Fusarium oxysporum.* The bacterial cultures were obtained from Pathology Lab, Bhagwan Mahaveer Cancer Hospital and Research Centre, Jaipur, and fungal cultures were obtained from microbiology lab, Department of Botany, University of Rajasthan, Jaipur.

All the bacteria were incubated at 30 ± 0.1°C for 24 hours by inoculation into Nutrient Broth (Sigma). Sterilized Petri dishes (9 cm diameter) were inoculated with 0.01 ml of one of the above culture media (10^5 bacteria per ml). Muller-Hinton agar (Sigma), sterilized in a flask and cooled to 45–50°C, was distributed by pipette (15 ml) into each inoculated Petri dish and swirled to distribute the medium homogeneously. Discs injected with extracts were applied on the solid agar medium by pressing slightly (Collins *et al.*, 1989, Bradshaw, 1992). The treated Petri dishes were placed at 4°C for 2 hours and then incubated at 35 ± 0.1°C for 24 hours.

The fungal strains were maintained on the Potato Dextrose Agar (HI-MEDIA) and stored at 4ºC. Cultures were reactivated before test. Potato Dextrose Agar plates were used for the activation and incubated for 16-18 hours at 37ºC. For inoculation *Aspergillus sp.* dried spores were distributed uniformly on the surface of agar plates with the help of a sterile cotton swab. *Fusarium oxysporum* was inoculated by taking a piece of fungal colony on a sterile cotton swab and gently swabbing on the surface uniformly. The fungal growth was checked after 24, 48 and 72h depending on the period of incubation time required for a visible growth; 48h for *Aspergillus niger, Aspergillus fumigatus*, 72h for *Aspergillus flavus* and *Fusarium oxysporum*

At the end of the period, inhibition zones formed on the medium were measured with a transparent ruler in millimeters and compared with the standard drugs prepared by using standard antibiotics as Ampicilin (10µg/ml), Streptomycin (10µg/ml), and Tetracyclin (30µg/ml) for Bacteria, and Amphotericin B (25µg/ml), and Ketoconazole (30µg/ml) for Fungi in sterile distill water. The experiment was performed in triplicate, and average diameter of zone of inhibition was obtained.

Phytochemical investigation by TLC

The detection of active principles in medicinal plants plays a strategic role in the qualitative and quantitative phytochemical investigation of crude plant extracts. TLC is a rapid and economical procedure for the determination of the main active principles of medicinal plants e.g., alkaloids, cardiac glycosides, coumarins, flavonoids, saponins, tannins, etc. TLC is also used for fractionation of the extract obtained by extraction procedure by using different solvent compositions.

The plant extracts were analyzed on silica gel layers with the aid of three solvent systems and six spray reagents, each one applied for the identification of active principles according to their polarity. Spots were visualized under short and long wavelength ultraviolet lights and, the plates were sprayed with a specific spray reagent.

The extent of the surface of the spot is a measure for the quantity of the material present (Pascual *et al.*, 2002). The volume of the spots applied on the chromatographic plates was 5µl, corresponding to approximately 300µg for each dry extract. Chromatography was performed in the following solvent systems: Nonpolar solvent: toluene-acetone (8:2); semi-polar solvent: toluene-chloroform-acetone (40:25:35); polar solvent: n-butanol-glacial acetic acid-water (50:10:40). The chromatograms were observed first without chemical treatment, under UV 254 nm and UV 365 nm light, and then using the spray reagents.

Determination of Minimum Inhibitory Concentration

For determination of Minimum Inhibitory Concentration (MIC), the method of Cheesbrough (2000) was used. Stock solutions were prepared by dissolving the extracts in DMSO. Two-fold serial dilutions were employed to determine MIC values. Each microorganism was incubated with an extract in duplicate tubes containing a total volume of 10 mL.

The final concentration of extract was in the range 0.1 to 1.5 mg/mL. Control tubes without extract were constituted similarly. Antibiotics were included as positive control in different tubes. The MIC was the lowest concentration of extract with no visible bacterial growth or no turbidity.

HIV-1 Protease inhibition assay (Jeffrey and Christine, 1997)

All enzymatic and non-enzymatic reaction were performed in reaction vial i.e. 1.25 ml. eppendorf tubes. The peptidolysis of the substrate peptide substrate- … (Ac-Arg-Lys-Ile-Leu*Phe-Leu-Asp-Gly-NH_2) by HIV-1 protease was carried out in 10% DMSO, 0.1M NaCI, 50 mM KOAc, pH- 5.5. Peptidolysis was initiated with nanogram quantities of HIV-1 protease and quenched by the addition of 50 µg of a carbamylating reagent; which consists of 0.6% KNCO and 10% DMSO in 0.2M K_2HPO_4, pH 7.0, and is freshly prepared before use. After 3h at ambient temperature the carbamylating reaction is quenched by 100µl of color mixture (antipyrine/H_2SO_4 reagent + oxime reagent). Color development is accomplished by a 16-h incubation in the dark at ambient temperature followed by a 24 min incubation at 45°C under a fluorescent light. The absorbance is determined at 480 nm by Kary 100 UV-Visible spectrophotometer. The background absorbance 0.1 to 00.3 caused by the Cyanate is automatically subtracted by taking it as a blank in reference tube. The free Phe was used as a standard for the both carbamylation and diacetylcarbamido reactions. All steady- state enzymatic data were analyzed manually. An standard curve was also plotted for enzymatic reaction (Fig.2).

3. Results and Discussion

In recent times ethnomedicinal and traditional pharmacological approaches are achieving great appreciation in modern medicine, because the search for new potential medicinal plants is often based on an ethnomedicinal origin (Muthu *et al.*, 2006). Plants face many stresses like diseases, pests, drought etc. in their life cycle and in the process to overcoming these stresses they produce secondary metabolites, which are not important for the metabolic functions of the plant but help to face the stressful conditions. Some of these secondary metabolites have capacity to fight microorganisms and can be used for medicinal purposes (Anon, 1994; Muthu *et al.*, 2006).

The ethnomedicinal study reveals that *E. tirucalli* is a plant of very high ethnomedicinal value and its different parts are used as medicines by the local traditional healers (Table 1). Among the different plant parts, the leaves are most frequently used for the treatment of various diseases. The methods of preparation fall into many categories like, plant parts applied as a paste (38%), juice extracted from the fresh plant parts (24%), and powder made from fresh or dried plant parts (20%), some fresh plant parts (6%), and decoction (12%). External applications (mostly for skin diseases, snakebites, and wounds) and internal consumption of the preparations were involved in the treatment of diseases. Latex is used for asthma, cough, earache, neuralgia, rheumatism, toothache, and warts. The latex is diluted in water and used internally for snakebite, as well as benign and cancerous tumors. This correlates with the phytochemical studies of Correia (1994) and Betancur-Galvis *et al.* 2002).

Table 1: Ethnomedicinal importance of *E. tirucalli* in Rajasthan

Part used	Disease treated	Mode of Administration
Latex	Asthma	Five ml diluted latex is administered twice a day.
	Cancer	Paste of Fresh leaves and latex is diluted with water and taken once a day
	cough	Latex diluted with water taken twice a day
	Ear problems	Latex is boiled in Mustard oil in ratio of 1:5, and 3 to 4 drops of this oil are dropped in affected ear twice a day
	Snake bite and scorpion sting	About two gm of latex with about 100ml water is taken orally within 2 hours of snakes bite.
	Toothache	A decoction obtained by boiling latex water in ratio of 1:6, This decoction is used as mouth wash twice a day
	Intestinal parasites	Dried latex and a fine paste of seeds are taken internally with Luke warm water after each meal.
	Skin problems	Fresh latex is applied on the affected area.
Leaves	Skin problems	Decoction of leaves applied on affected skin externally.
	Nose ulcers and hemorrhoids	Poultice of the root or leaves is used for nose ulcers and hemorrhoids.
Stems	Thorn Extraction	The powdered stems are used as a poultice to extract thorns.
	Swelling	Crushed stem are applied to swellings
	leprosy and paralysis	Wood decoction is used for leprosy and paralysis of the hands and feet after childbirth.
	colic and gastric problems	decoction of the branches is used for colic and gastric problems
Root	Rheumatism	Fresh root or latex and 'Asgandh' are taken in equal quantities and ground to a fine paste. Two gm Paste mixed with 5 gm honey is administered orally twice a day

Table 2: Estimation of phytochemical constituents by TLC

S. No.	Phytochemical	Spray reagents	Light used	Observation	Presence in *E. tirucalli*
1.	Alkaloid	Dragendorff reagent	Visible light	Stable orange color	++
2.	Flavonoids	After spraying with PEG	UV 365 nm	-	-
3.	Saponin	Liebermann-Burchard reagent	Visible light	Green, red-brown, violet or blackish zone.	-
			UV 365 nm	Green fluorescence.	-
4.	Coumarins	5% KOH	UV 365 nm.	Blue green color	+
5.	Ployphenols and Tannins	3% FeCl3	Visible light	Dark zones	+
6.	Cardiac glycosides	Liebermann-Burchard reagent	Visible light	-	-
7.	Triterpenes	Liebermann-Burchard reagent	Visible light	Red-brown zones	+++

The phytochemical estimation was done using Thin Layer Chromatography analysis of leaf extract of *E. tirucalli*. Results are presented in Table 2. In *E. tirucalli*, triterpenes were present in high concentration, alkaloids were present in average amount, and cardiac glycoside, poly-phenols, flavanoids and tannins were present in small amount, while saponin, and coumarin were not reported in this study.

Table 3 shows the antimicrobial activity of the methanolic extracts from the zone of inhibition produced by the extracts. It was observed that *E. coli,* and *P. aeruginosa* were most sensitive to the leaf extract while *K. pneumoniae* and *S. aureus* were least sensitive to the methanolic leaf extract. Stem bark extract exhibited significant antimicrobial activity against *P. vulgaris, K. pneumonia* (Fig.1). The results of antimicrobial activity were consistent with previous reports (Dekker *et al.,* 1983; Tomas-Barberan *et al.*, 1990; Akihisa *et al.*, 2002) on related Euphorbia species against Gram-negative bacteria. Unlike Gram-positive bacteria, the lipo-polysaccharide layer along with proteins and phospholipids are the major components in the outer surface of Gram-negative bacteria (Ferreira *et al.*, 2001).

Table 3. Inhibition zone showing antimicrobial activities of Standard drugs and different extracts of *E. tirucalli* L.

Extracts	Leaf extract			Stem bark extract			Strepto.*	Amp.	Tetra.	Ampho. B	Keto.
Organisms	2.5 mg	5 mg	7.5 mg	2.5 mg	5 mg	7.5 mg	10 µg	10µg	30µg	-	-
B. subtilis (ATCC 6633)	9.00 (46.39)#	11.5 (59.27)	12.5 (64.74)	8.5 (43.81)	13.5 (69.88)	17.0 (87.62)	19.4 (100)	15	-	-	-
E. coli (ATCC 25922)	9.5 (46.11)	17.0 (82.52)	19.5 (94.66)	9.0 (43.68)	14.0 (67.96)	18.5 (89.80)	20.6 (100)	-	27	-	-
E. coli (clinical isolate)	6.3 (34.42)	8.6 (46.99)	13.2 (72.13)	7.2 (39.34)	9.6 (52.45)	16.3 (89.07)	18.3 (100)	18	25	-	-
P. vulgaris (ATCC 13315)	8.0 (35.55)	15.5 (68.88)	17.5 (77.77)	12.6 (56.0)	14.5 (64.44)	21.5 (95.55)	22.5 (100)	-	20	-	-
P. aeruginosa (ATCC 9027)	7.0 (35.00)	16.0 (80.00)	18.0 (90.00)	8.0 (40.00)	9.4 (47.00)	14.5 (74.74)	20.0 (100)	14	12	-	-
P. aeruginosa (clinical isolate)	5.3 (29.60)	12.1 (67.59)	13.6 (75.97)	6.6 (36.87)	8.1 (45.25)	13.5 (75.41)	17.9 (100)	15	-	-	-
S. aureus (ATCC 6538P)	6.0 (30.76)	8.5 (43.58	18.79 (96.41)	9.5 (48.71)	14.0 (71.79)	16.5 (84.61)	19.5 (100)	16	-	-	-
S. aureus (clinical isolate)	6.3 (33.87)	5.1 (27.41)	16.8 (90.75)	8.3 (44.62)	12.1 (65.05)	15.3 (80.80)	18.6 (100)	-	24	-	-
S. enteritidis (clinical isolate)	6.5 (31.86)	10.0 (49.01)	16.5 (80.88)	10.6 (51.96)	15.0 (73.59)	18.0 (88.23)	20.4 (100)	17	26	-	-
K. pneumonia (ATCC 13883)	5.5 (28.20)	9.0 (46.15)	11.8 (60.51)	9.5 (48.71)	14.0 (71.79)	18.0 (92.30)	19.5 (100)	17	-	-	-
C. albicans (ATCC10231)	5.6	8.2	9.9	4.1	7.6	16.2	-	-	-	23	19
C. albicans (clinical isolate)	5.9	9.4	10.6	5.2	8.1	15.6	-	-	-	21	25
C. tropicalis (clinical isolate),	5.1	8.3	11.6	4.3	7.6	9.1	-	-	-	-	21
A. flavus (Lab isolate)	1	2.6	4.2	1.3	3.1	4.8	-	-	-	19	21
A.niger (Lab isolate)	1.1	2.3	3.8	1.2	2.4	3.6	-	-	-	22	-
A. fumigatus (Lab isolate)	1.5	2.6	4.6	1.2	2.3	4.6	-	-	-	21	24
F. oxysporum (Lab isolate)	0.6	1.6	4.2	1	2.1	3.1	-	-	-	25	na

Diameter of zone of inhibition in mm,

#Figures in parenthesis indicate percentage diameter inhibition, and the results shown are the mean of three replicates

*Inhibition zone of Streptomycin is considered as 100% to compare the extract efficacy in respect to standard antibiotics

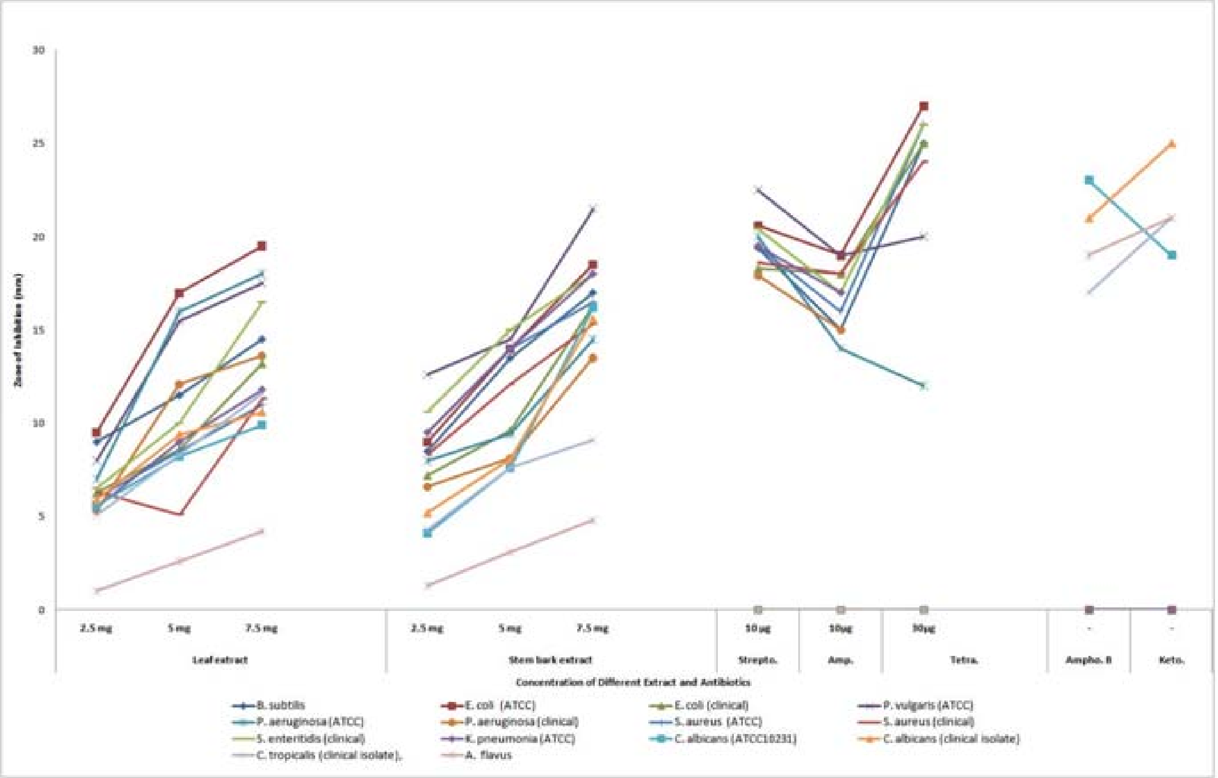
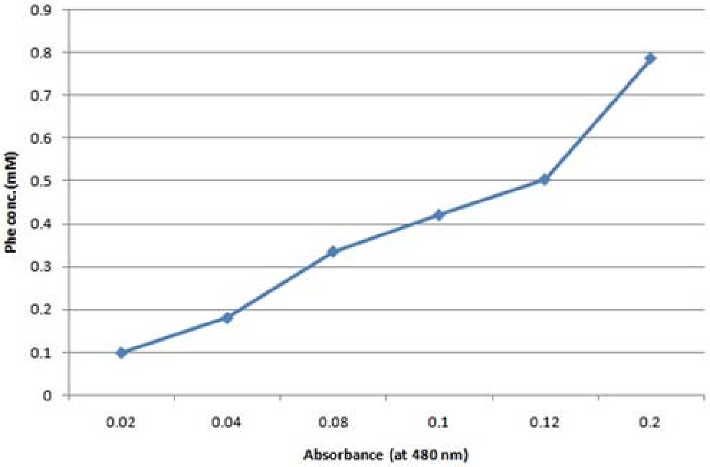


Fig. 2. Standard curve of enzymatic reaction of anti HIV activity



The outer lipo-polysaccharide layer of cell wall slows down the accessment of most phytochemical compounds to the peptidoglycan layer. This is the cause why the Gram-negative strains have resistance to the toxic effect of plant extracts exhibiting antimicrobial activity. Infections caused by *S. aureus* are among the most difficult to treat with conventional antibiotics (Sueller and Russell, 2000).

Similar observations were made by Kuhnt *et al.*, (1994), Meyer and Afolayan (1995) and Saxena *et al.*, (1996) while studying the antimicrobial activity of *Hyptis berticillata*, *Helichrysum aureonitens* and *Moneses uniflora*, respectively. The weak activity shown by the acetone extract against the Gram negative bacteria could be due to the presence of compounds in the extract possessing lipophilic characteristics as suggested by Lall and Meyer (Lall and

Meyer, 2000). These observed antimicrobial properties agree with its use in traditional medicine. Traditionally, extracts of the plant are used in sore and wound healing, as eardrop for boils in the ear and treatment of boils. They are also used in the control of diarrhea and dysentery (Ajali *et al.,* 2002; Annapurna *et al.,* 2004).

The large zones of inhibition exhibited by the extract against *S. aureus* and *B. cereus* justified their use by traditional medical practitioners in the treatment of sores, bores, and open wounds (Parekh and Chanda, 2005). In addition, the moderate growth inhibition against *E. coli* justifies its use in the control of diarrhea and dysentery. *E. coli* is the common cause of traveler's diarrhea and other diarrheagenic infections in humans. From table 4 is clear that low MIC exhibited by the extract against *S. aureus* is of great significance in the health care delivery system, since it could be used as an alternative to orthodox antibiotics in the treatment of infections caused by these microbes, especially as they frequently develop resistance to known antibiotics (Lin *et al.,* 2002).

Table 4: Determination of Minimum Inhibitory Concentration (MIC)

S.No.	Tested organisms	MIC (mg/mL.)
1.	*B. subtilis (*ATCC 6633)	0.1
2.	*E. coli* (ATCC 25922)	na
3.	*E. coli* (clinical isolate)	0.1
4.	*P. vulgaris* (ATCC 13315)	0.25
5.	*P. aeruginosa* (ATCC 9027)	0.5
6.	*P. aeruginosa* (clinical isolate)	0.2
7.	*S. aureus* (ATCC 6538P)	1.0
8.	*S. aureus* (clinical isolate)	0.2
9.	*S. enteritidis* (clinical isolate)	>0.1
10.	*K. pneumonia* (ATCC 13883)	0.2
11.	*A.flavus*	0.5
12.	*A.niger*	0.2
13.	*A.fumigatus*	0.5
14.	*F.oxysporum*	>1

Table 5: Results of Anti-HIV activity

S. No.	Plant source	Absorbance (OD at 280 nm)
1	OD of *E. tirucalli extract*	0.5386 ±0.005
2	OD of (+) control	0.00030±0005
3	OD of (-) control	0.65230±0005

Table 6: standard curve of enzymatic reaction

Phe conc.(mM)	Absorbance (at 480 nm)
0.02	0.0989 ±0.005
0.04	0.1803 ±0.005
0.08	0.3351 ±0.005
0.1	0.4206 ±0.005
0.12	0.5036 ±0.005
0.2	0.7862 ±0.005

Parasitic fungi cause many different diseases, which may be superficial, subcutaneous, or deep inside man and animals. In the superficial mycoses, the fungus is limited to the horny layer of the skin and to structures derived from it, while in the subcutaneous and deep mycoses there is a deeper invasion of the tissues (Laks and Pruner, 1989; Kwon-Chung and Bennett, 1992).

From table 3, Fig. 1 is clear that 7.5 mg/ml of leaf extract showed maximum antifungal activity with *A. fumigatus* with inhibitory zone of 4.6 mm diameter, which was followed by *F. oxisporum* and *A. flavus* at inhibitory zone of 4.2 mm diameter at 7.5 mg/ml extract concentration. In case of stem bark extract of *E. tirucalli* was observed, maximum inhibiton was shown by *A. flavus* (4.8 mm), followed by *A. fumigatus* (4.6 mm). In terms of Minimum Inhibitory Concentration, minimum MIC was recorded in case of *A. niger* (0.2 mg/ml), which showed a less potential activity than *E. hirta*. MIC against *A. flavus* and *A. fumigatus* showed MIC of 0.5 mg/ml, and more than 1.0 mg/ml. MIC was showed by *F. oxysporum* (Table 4).

The various types of secondary metabolites are known to possess antimicrobial activities. These products may exert their action by resembling endogenous metabolites, ligands, hormones, signal transduction molecules, or neurotransmitters and thus have beneficial medicinal effects on humans due to similarities in their potential target sites. Flavonoids are found to be effective antimicrobial substances against a wide range of microorganisms, probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall; more lipophilic flavonoids may also disrupt microbial membrane.

Phenolics and polyphenols present in the plants are known to be toxic to microorganisms. Antimicrobial activity of tannins may be related to their ability to inactivate microbial adhesions, enzymes and cell envelope transport proteins, they also complex with polysaccharides. Many plant genetic resources have been analyzed for their active constituents possessing antimicrobial activities. The broad-spectrum antimicrobial activity exhibited by *E. tirucalli* may be attributed to the various active constituents present in it, which either due to their individual or combined action, exhibit antimicrobial activity. Hence, the present findings provide a scientific base for some of the medicinal claims of *Euphorbia tirucalli.* Considering these facts; traditional medicines and medicinal plants obviously represent a great source of novel leads for drug development.

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