INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online atwww.ijpcbs.com

Research Article

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF TUBER EXTRACTS OF *EUPHORBIA FUSIFORMIS* VAR. *KHANDALLENSIS* (BLATT. &HALLB.)BINOJK.&N.P.BALAKR

Sanjay Jagtap[1]* and Rajendra Satpute[2]

[1]Elphinstone College, Mumbai, Maharashtra, India.
[2]Govt.Institute of Science, Aurangabad, Maharashtra, India.

ABSTRACT

Euphorbia fusiformis(Synonym: *Euphorbia acaulis*)is a rare medicinal herb belonging to family Euphorbiaceae. Due to indiscriminate collection from naturalhabitat it has become endangered.The dried root powder and the fresh rhizome are used to increase the secretion of the mother's milk, while the tuber latex is applied to heal chronic wounds, skin diseases, liver disorders and diarrhoea. In the present study, phytochemical, antimicrobial and antioxidant activityof the tuber extracts of *Euphorbia fusiformis* were evaluated. Phytochemical screening indicated thattubers are rich in a variety of primary and secondary metabolites such as carbohydrates, alkaloids, vitamin C, vitamin E, flavonoids, phenols, glycosides, saponins and minerals. Present investigation showsDPPH anti-scavenging activity in organic extracts such as methanol (56.47%), chloroform (10.23%), acetone (16.51%) and water (76.92%). The antimicrobial activity of tuber extracts against clinically isolated microbes like *Staphylococcus aureus, Pseudomonas aeuroginosa, Salmonella typhi, Escherichia coli* and *Aspergillusniger.* The study highlights the phytochemical and ethno-pharmacological significanceof *Euphorbia fusiformis.*

Keywords: Phytochemical, antimicrobial, ethno-pharmacological, antioxidants.

INTRODUCTION

Euphorbia fusiformis (Synonym: *Euphorbia aqualis*) is a rare medicinal plant belonging to family Euphorbiaceae found in Andhra Pradesh, Bihar, Goa, Gujarat, Karnataka, Maharashtra, Rajasthan, Tamil Nadu, Uttar Pradesh, West Bengal,. In Gujarat state, it is found in Dangs, Rajpippala and Chota Udaipur regions. In Maharashtra it is found in parts of Western Ghats at Khandala, Lonawala, Bhimashankar in Pune, Panchagani, Mahabaleshwar in Satara, Karjat in Raighad and Konkan region. (Cooke, 1906;Blatter and MaCann, 1931; Hooker, 1887;Duthie, 1994; Gamble, 1921; Shah, 1978; Britto, 2002).

*Euphorbiafusiformis*has one of the largest tuberous root stocks in this genus. The species epithet refers to its fusiform root. Locally it is called "Ban-Muli" by the tribal people. It is said to be of medicinal value, its latex being used as an antidote for snake and scorpion bites. The tuber pulp is used as a cure for arthritic pains in some regions of the Himalaya(SoumenAditya,2010).The ethnobotanical value of this plant is due to its action as a remedy for several diseases like rheumatism, gout, paralysis and arthritis(Prakash, *et al.,*2001),liver disorders and diarrhoea (Raju,*et al.,*2004).The tuberous roots of this plant were used by *Bhagats* (tribal physicians) of Dangs forest for the treatment of various abdominal disorders, especially for tumors of abdomen, and urinary stones. However, after extensive literature search, we came to know that only few pharmacological studies have been carried out on this plant, namely, its anti-inflammatory(Singh, *etal.,*1984) and antibacterial activities(Natrajan, *et al.,* 2005, Ramchandran, *et al.,*2008).

The dried root powder and the fresh rhizome are used to increase the secretion of the mother'smilk, while the rhizome latex is applied to heal chronic wounds, skin diseases, liver disorders and diarrhoea. A paste from the leaves is applied to the forehead to get relief from acute headaches (Raju, *et al*., 2004).Paste of tuberous rootstock is applied locally in rheumatic pain. In case of gout the paste of tuber is warmed and then administrated locally (Swarnkar, *et al.,* 2008).

Various classes of chemical constituents have been isolatedfrom different species of Euphorbiaceae. From *Euphorbiaretusa* flavonol glycosides like quercetin-3-glucoside,quercetin-3-glucouronide, quercetin-3-rhamnoside andquercetin-3-rutinoside were isolated while in a recent study, apentacyclictriterpenebetulin, the steroid â-cytosterolandnumber of fatty acids. All the members of this family contain skin irritating and tumor promoting diterpenoids (Evans andTaylor, 1983). Some species are used to cure diarrhoea,migrains, intestinal parasites and warts (Singla and Pathak,1990).

Euphorbia hirta contains flavonoids, terpenoids, phenols, essential oil, Alkaloids, saponins, amino acid and minerals(Asha, *et al.,*2014).

In present study the phytochemical, antimicrobial, antioxidant activity and flavonoids analysis of the tuber extracts of *Euphorbia fusiformis* were evaluated.

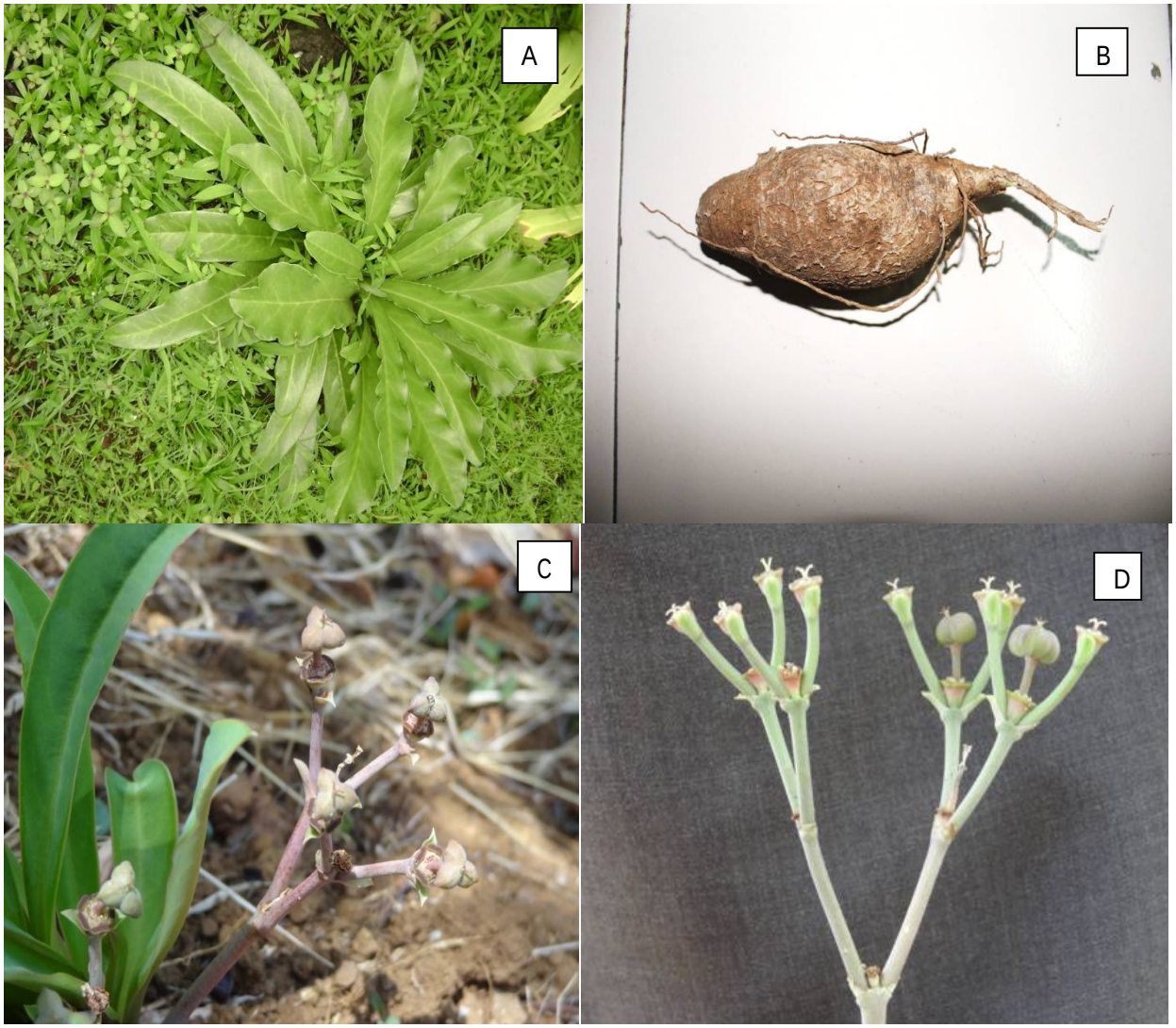


Fig.1: *Euphorbia fusiformis*

A: Habit B: Tuber

C: FruitsD: Inflorescence

MATERIALS AND METHODS

Sampling

Fresh samples of tubers of *Euphorbia fusiformis*were collected from Lonawala, Dist. Pune region of Western Ghats of Maharashtra (Fig.1A to D). These plants were identified and authenticated using herbarium collection at Department of Botany, DST-FIST School of Life Science, SRTM University, Nanded (MS), India and Department of Botany Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. Fresh tubers were washed thoroughly under running tap water followed by sterile distilled water and dried under shade. The shade dried material was ground into coarse powder using mechanical grinder. This coarse powder was sieved by 1 mm pore size sieve. The powder was stored in airtight containers at room temperature till further phyto-chemical screening of secondary metabolites.

Soxhlet extraction

Exhaustive Soxhlet extraction was performed using a classical Soxhlet apparatus with accurately weighed 10 g of the crude powder of plant material for 18-40 h. The solvents like water, methanol, chloroform and acetone were used for extraction. The extraction was conducted for 6-8 h /d and finally all the extracts were evaporated under vacuum. The water, methanol, chloroformand acetone extracts of tubers of the plant were prepared according to standard methods (Harbone, 1998). Nitrogen gas was purged through these extracts to prevent oxidation of secondary metabolites. These extracts were sealed in airtight containers and stored at -4^{0}C.

Phyto-chemical screening

Phyto-chemical screening of active plant extracts was done by following the standard method of Khandelwal (2000), for the qualitative analysis of various phytochemicals such as alkaloids, carbohydrate, glycosides, saponins, flavonoids and phenols which could be responsible for anti-oxidant activity.

Mineral analysis

Micro-scaled digestion

CEM-MARS 6 microwave oven was used for micro-scaled digestion. 0.5 g of herbal samples were weighed and transferred to CEM-Xpress vessels. About 8-10 mL of conc. HNO_3 was added to the samples. The samples were pre-digested for 10-15 min prior to capping the vessels. The CEM-Xpress vessels were assembled for microwave irradiation. The microwave program was adjusted with respect to the number of vessels and reference to the guidelines of CEM at 1000 W with 100% level. 25 minutes ramping period was used to reach the digestion temperature of 180^{0}C which there upon was maintained for 15 min. The CEM-Xpress vessels were kept in fume hood for cooling and to release the pressure by uncapping. The contents were transferred to 50 mL volumetric flasks and volume was made with distilled water. The solutions were filtered prior to use.For calibration, Leeman and Thomas Baker Std. sample were used as the reference for the calibration range.The spray chamber, nebulizer and torch assembly was completely cleaned to eliminate contamination. The plasmawas stabilized for 15 min by flushing with distilled water.

An Instrument Calibration was performed to check the wavelength shift and the same was successful with a minimum deviation of <10 % with master scan. After calibration, the instrument was optimized with 10 PPM solution containing elements (Table 1) and the same as optimized with maximum intensity and best BEC at the parameters mentioned in operating condition above.

Diluted samples were used for further analysis by using Teledyne Leeman, ICP (Induction Coupled Plasma).

Table 1: Instrumental characteristics and setting for ICP-OES: Spectrometer LEEMAN LAB's Simultaneous ICP-OES PRODIGY XP Dual System

	Parameters range		Actual Parameters
	Min	Max	
Power	0.1	2.0	1.1 KW
Coolant flow	5	20	18 L/Min
Auxiliary flow	0.0	2.0	0.2 L/M
Nebulizer flow	5	60	34 psi
Plasma torch	--	--	Dual
Spray chamber	--	--	Cyclonic
Nebulizer	--	--	Concentric
Sample aspiration rate	0.5	2.0	1.4mL/min
Replicate read time	--	--	40 sec per replicate for Axial

Determination of Vitamin E by HPLC

Standard preparation

dl α-tocopherol acetate (96%), (Vitamin E) manufactured by Merck was used for calibration of standard curves. About 1 mg of dl α-tocopherol acetate was dissolved in 1 mL in HPLC grade methanol. Dilutions of 100, 50, 25, 10 µg/mLwas prepared and the pre-treated sample extracts and stock solutions were filtered through 0.45 µm syringe filters.

Reverse phase HPLC method

The concentration of -tocopherol (Vitamin E) in the extracts was determined by Agilent Technologies 1200 series Quaternary system, equipped with auto sampler, quaternary pump, degasser, column oven, and a DAD detector. The spectral data was collected at UV detection at 220 nm. The solvent system of acetonitrile and water (95:5) was used a gradient mobile phase on Agilent ZORBAX 300 SB column (4.6 × 150 mm × 5 µm) at a flow rate of 1.0 mL/min, 10 µg injection volume and detection was optimized at 220 nm with 15 min separation time.

Vitamin C

About 0.25% ethanolic solution of DCPI (2, 6-dichlorophenol-indophenol sodium salt) was prepared for the detection of Vitamin C. To 0.5 mL of sample extracts, 2 drops of DCPI indicator was added. The blue coloration changed to red confirmed the presence of vitamin C. The test was carried out for all the extracts (British Nutrition Foundation, 2004).

Anti-scavenging activity

DPPH solution (0.1 mM) was prepared in methanol by dissolving 0.0394 g DPPH in 1000 mL methanol. The solution was kept in darkness for 30 min to complete the reaction. The free radicals scavenging activity of the crude extracts was determined by the 1, 1-diphenyl-2-picryl-hydrazil (DPPH). The antioxidant activity was measured by the standard method described by Brand-Willium, *et al.,* (1995) wherein, the bleaching rate of stable free radical DPPH was monitored at a characteristic wavelength in the presence of the sample. In its radical form, DPPH absorbed at 570 nm, but upon reduction by an antioxidant or radical species its absorption decreased. The capability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging effect (%) = (ABS $_{control}$- ABS $_{sample}$) / (ABS $_{control}$) X 100),

whereas ABS $_{control}$ is absorbance of negative control and ABS $_{sample}$ is the absorbance of the reaction mixture containing the sample extract.

In vitro anti-microbial activity

Plates were prepared for the assay by using standard pour plate technique for bacterial cultures. About 0.1 mL of bacterial suspension was spread onto the Nutrient Agar medium. Clinically isolated microbial strains *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Staphylococcus aureus and Aspergillus terreus*were used.A spore suspension of *Aspergillus* was prepared in sterile distilled water which was added with Tween-20 as a surfactant. 0.1 mL of spore suspension was spread onto the Potato dextrose Agar medium. These plates were further used for the assay purpose immediately.

Paper discs approximately 6 mm in diameter were carefully cut from multiple layers of thin filter paper with a sharp cork borer. Sterilization of the discs was done by autoclaving them at 121^{0}C for 15 min and was allowed to dry before use. Hot-air sterilization reduces the absorptive capacity of the paper, but drying for 1 h at 100°C had little effect. Paper disc was grasped with finely pointed forceps, and its lower edge was touched to the surface of the agar plate. Fixed amount (10 µL) of test solution and standards were used to transfer onto the paper disk by Micropipette. Clean and Sterile forceps were used for each respective solution or the forceps were flamed and cooled between the changes of samples. The discs were kept on the agar to allow adequate room for the development of the zones of inhibition. Two discs were introduced on 90 X 15 mm petri dishes. Reference marks on the outside of the petri dishes were given for identifying the discs.

The plates were incubated at 37^{0}C for 24 h. Results were recorded in the terms of zones of inhibition in mm. The fungal culture plate was incubated at 28^{0}C for 6 d.

Flavoinoids analysis by HPTLC

The standards Quercetin, Kaempferol, Catechin gallate, Rutin hydrate and Hesperidin were procured from Sigma Aldrich USA. All the standard solutions were prepared in ethanol whereas hesperidin in water.

Chromatography was performed on silica gel 60F_{254} (10 cm X 10 cm; 25 mm layer thickness; Merk) with aqueous, methanolic, chloroform and acetone extracts of *Euphorbia fusisformis* tuber. The fraction residues were collected and (10 µL) subjected for HPTLC (CAMAG, Switzerland)

analysis. The fractions were impregnated on silica gel 60F_{254}TLC plate. The plate was air dried and then inserted in CAMAG-twin through lass chamber containing solvent system of composition with ethyl acetate, acetic acid, formic acid and water (100:11:11:27) as a gradient mobile phase for 20 min. The well eluted TLC plate was then dried at 105^{0}C for 15 min and scanned using Scanner 3 (CAMAG, Switzerland) at 254 and 366 nm using Win Cat 4 software.

RESULTS AND DISCUSSION

Optimization of extraction method

In order to extract the phytochemicals from herbal samples efficiently, variables involved in this procedure were optimized, including extraction solvent (Water, Methanol, Chloroform, Acetone, IPA, 100%), extraction method (Soxhlet, reflux, percolation), and extraction time (18-40 h). The extraction time in water was 40 hours. The biomass was refluxed for 40 hours, and then it was dried naturally for 2-3 days. To the dried biomass, 100% methanol was added and the reaction was percolated to extract phytochemicals. The methanolic fraction was collected in amber coloured bottle under nitrogen atmosphere. The material was dried for 5-6 h. The procedure was repeated for chloroform, acetone and IPA. The extraction time was optimized for all the samples. All the extracts were preserved under nitrogen atmosphere in amber coloured bottles at -4^{o}C.

Phyto-chemical screening

It is known that plants are rich in a variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides, saponins and volatile oils. It is necessary to identify the phytochemical components of local medicinal plants usually employed by herbalists in the treatment of diseases (Banso and Adeyemo, 2007).The presence or absence of certain phytochemicals could be used to explain some of the biological activity of certain plant extracts. For example, saponins are a special class of glycosides which have soapy characteristics and have been reported to be active anti-fungal agents. Anti-microbial properties of a number of tannins, flavonoids, alkaloids have been reported. Not only the anti-microbial properties have been ascribed to these plant phytochemicals, but other biological activities including modulation of the immune system have been assigned to these compounds in plants.

Phyto-chemical screening of the tuber extracts of *Euphorbia fusiformis* revealed the presence of different phytochemicals. Indeed, phytochemical investigations of the plant *Euphorbia fusiformis*have resulted in occurrences of carbohydrates, alkaloids, glycosides, saponins, flavanoids, tannins, phenols, vitamin E and vitamin C.

Table 2 illustrates the results of phytochemical screening of all the extracts of *Euphorbia fusiformis*. The qualitative analysis of carbohydrates (Benedict's reagent test) and glycosides (Borntranger's reagent) were carried out in all extracts i.e. aqueous (S1), methanol (S2), acetone (S3) and chloroform (S4) extracts of *Euphorbia fusiformis*. The solutions turned red and pink confirmed the presence of carbohydrates and glycosides respectively. The hydrophilic carbohydrates and glycosides were present in water (S1) whereas and hydrophobic carbohydrates and glycosides were detected in rest of the organic solvents (S2-S4).

Table 2: Preliminary phyto-chemical screening of tuber extracts of *Euphorbia fusiformis*

Species Name	Ext.	Car	Sta	Prot	Glyc	Alk	Sap	Tan	Flav	Phe	Vit. C	Vit. E
Euphorbia fusiformis	WE	+++	-	-	++	-	++	-	++	+	+	-
	ME	++	+++	+	+	+++	+	GT +	+++	++	-	-
	CE	++	-	-	+	-	-	-	+	-	-	++
	AE	++	-	-	+	-	-	-	+	-	-	-

Ext = Extract, Car = Carbohydrate, Sta = Starch, Prot = Protien, Glyc = Glycoside, Alk = Alkaloids, Sap = Saponins, Tan = Tannin, Flav = Flavonoids, Phe = Phenols,
Vit C = Vitamin C, Vit E = Vitamin E, WE = Water Extract, ME = Methanol Extract, CE = Chloroform Extract, AE = Acetone Extract, GT = Gallotannins, PT = Pseudotannins.

+ = Significant, ++ = Moderate, +++ = Very good

The Mayer's test of extracts S2, S3, S4 displayed appearance of white turbidity for alkaloids. The alkaloids were absent in S1, S5 extracts of *Euphorbia fusiformis*. The dark brown coloration test for phenols was observed in S2-S5. No traces of phenols are found in water. The extracts S1-S4 were shaken with distilled water. The persistence of froth in S1 and S2 was observed, indicated the presence of saponins. The hydrophilic flavonoids were detected in extract S1. The water soluble vitamin C was found in *Euphorbia fusiformis*the vitamin E was qualitatively and quantitatively analyzed by HPLC method in tuber extracts S2, S3, S4 of *Euphorbia fusiformis*. The ethanol extract of *Euphorbia fusiformis* tubers have potential therapeutic value in the treatment of liver disorders and is safer to use even at higher doses when taken orally (Anusaya, *et al.,*2010).

Microwave digestion and mineral analysis: Optimization and calibration of Euphorbia fusiformistuber extracts

Iron and copper are of great importance for life. As redox-active metal, they are involved in photosynthesis, mitochondrial respiration, nitrogen assimilation and hormone biosynthesis.

Manganese is essential for plant metabolism and development and occurs in oxidation states II, III, and IV in approximately 35 enzymes of a plant cell. Zinc is important as a component of enzymes for protein synthesis and energy production and maintains the structural integrity of bio-membranes. Most of the zinc enzymes are involved in regulation of DNA-transcription, RNA-processing, and translation. Although the essentiality of Se to plants has not been established yet, Se is considered as a beneficial element in promoting plant growth in some plant species.

The focal point of our study was to develop effective digestion method for the preparation of mineral analysis by ICP. Microwave digestion is a common technique used by elemental scientists to dissolve heavy metals in the presence of organic molecules prior to analysis by inductively coupled plasma, atomic absorption, or atomic emissionmeasurements(Kingston *et al.,* 1988).

Quantitative multi-elemental analysis by inductively coupled plasma (ICP) spectrometry depends on a complete digestion of solid samples. However, fast and thorough sample digestion is a challenging analytical task which constitutes a bottleneck in modern multi elemental analysis. Additional obstacles may be that sample quantities are limited with low elemental concentrations. In such cases, digestion in small volumes with minimum dilution and contamination is required in order to obtain high accuracy data.

After optimization, a new calibration method was created for measuring these samples; the wavelengths used for calibration were Cu 324.754 nm, Mn 257.610, Se 196.090, Fe 259.940, and Zn 213.856. Calibration STD solutions were measured 3 times one by one with an RSD < 1%. Once all the calibration standards are finished, a necessary background correction was applied for each wavelength. The results are depicted in Table 3.

We have developed a micro-scaled microwave digestion procedure and optimized it for accurate elemental profiling of plant materials. A commercially available 40- position rotor with 5 mL Poly tetraflouro ethylene (PTFE) vials, originally designed for microwave-based parallel organic synthesis, were used as a platform for the digestion. The novel micro-scaled method was successfully validated by the use of various certified reference materials (CRM). The micro-scaled digestion procedure was applied on crude powder of dried plant material in small batches.

We have determined the 5 elements in aqueous extract given in Table 2. Thereby, the concentration of minerals in plant extracts had the different profiles and quantitative differences had been detected.

The most abundant microelement was Fe in *Euphorbia fusiformis*whereas, copper was found at the lowest concentration. The content of iron was especially high in comparison to Zn, Cu, and Mn and, in consequence, the Se was not detected. The concentration of Zn content was less abundant. Vitamin E, vitamin C, carotenoids, Se and other trace minerals are important antioxidant components of animal diets and their roles in animal health and immune function are indispensable.In addition, several metallo enzymes which include glutathione peroxidase (Se), catalase (Fe), and superoxide dismutase (Cu, Zn, and Mn) are also critical in protecting the internal cellular constituents from oxidative damage. Only when these metals are delivered in the diet in sufficient amounts can the animal body synthesize these antioxidant enzymes. In contrast, deficiency of those elements causes oxidative stress and damage to biological molecules and membranes (McDowell, 2007).

Table 3: Accuracy of elemental concentrations in *Euphorbia fusiformis* after micro-scaled digestion expressed in ppm

Elements	*Euphorbia fusiformis*
Zn	10.0288
Cu	9.2312
Mn	56.1546
Fe	2711.736

Determination of Vitamin E by HPLC

Vitamins are a diverse group of organic compounds essential in trace amounts for the normal growth and maintenance of life. To ensure the adequate intake of vitamins, the human diet can be completed with a high range of multivitamin tablets and food products enriched with vitamins, in other words, these compounds are usually administered as nutraceuticals or functional ingredient. They are classified as either water-soluble or fat soluble. Vitamin E is fat-soluble whereas Vitamin C is water-soluble. Vitamin E is a generic term for tocopherols and tocotrienols, and it is fat-soluble antioxidant that blocks the production of reactive oxygen species formed when lipids undergoes oxidation. We employed reverse phase HPLC-analytical tool for qualitative estimation of vitamin E, in which HPLC has been coupled with UV detector.

Optimisation of HPLC method

To meet the requirements for quantitative analysis, various HPLC parameters were examined, including different columns (Agilent SB-C18 length 250 mm and 150 mm, width 4.6, particle size 5µm), column temperature (25^{0}C), and UV wavelength (220 nm). The best chromatographic resolution was obtained on Agilent SB-C18 length 4.6 X 150 mm, 5 µm column at 25^{0}C. The UV detector was monitored at 200-380 nm for fingerprinting analysis because the peaks were observed under this wavelength. The high intense peak was observed at 220 nm.

Method validation and calibration:

A calibration curve is simply a graph where concentration is plotted along the x-axis and area is plotted along the y-axis. (Response, absorbance, intensity, peak height, etc.) The line represents the calibration curve. Figure showed a calibration curve of vitamin E. We have constructed a calibration curve for vitamin E.

It was created by running 4 different calibration standards (10, 25, 50, 100 µg/mL). Each concentration gave a peak area (287.717, 761.253, 1594, 3023.3) respectively. Peak area was then plotted against the concentrations. The linear trend line has been drawn and linear regression equation has been calculated as y = mx + C. whereas, y = Area under the peak or Response, m = Slope of the linear line (Constant), x= Concentration in mL and C=intercept (Constant)

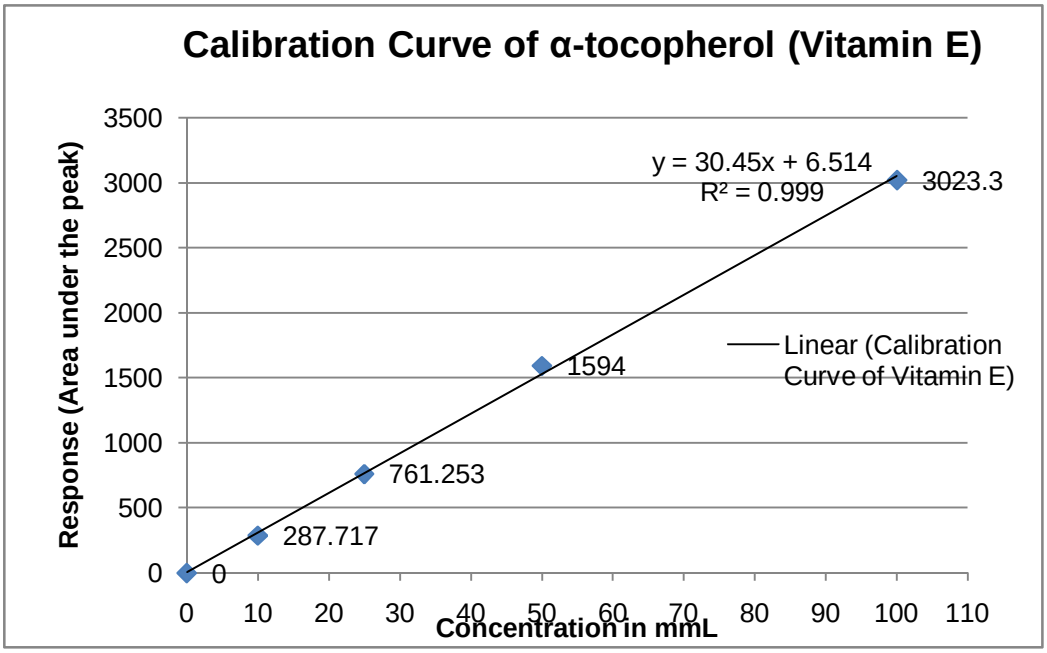


Fig. 3: Calibration curve of dl α-tocopherol acetate (96%) (Vitamin E)

HPLC is most widely used technique to analyzetocols, and both normal-phase (NP) and reversed-phase (RP) chromatography are applied (Kamal-Eldin*et al.,* 2000; Abidi, 2000; Ruperez, 2001). Vitamin E functions as a chain-breaking antioxidant, neutralizing free radicals and preventing oxidation of lipids within membranes (McDowell, 2000). The lipophilic vitamin E has been detected in the chloroform extract of *Euphorbia fusiformis*. The organic tuber extracts of *Euphorbia fusiformis*displayed significant anti-oxidant activity, proposed that the concentration of vitamin E might be higher along with the other natural anti-oxidants.

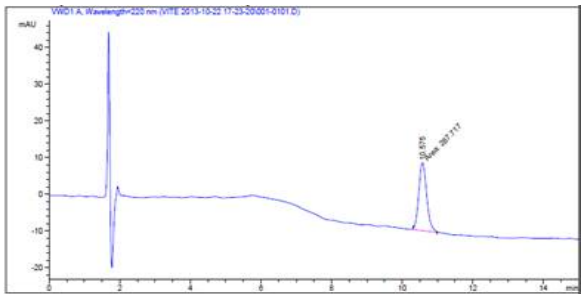


Fig. 4: Vit.E standard peak at 10µL

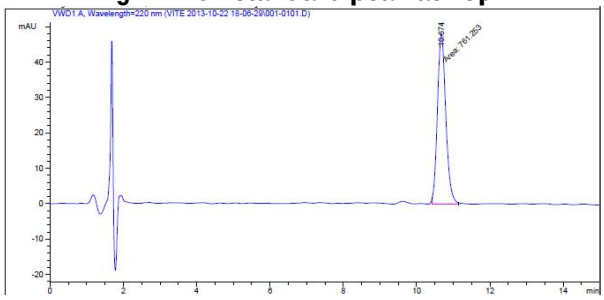


Fig. 5: Vit.E standard peak at 25µL

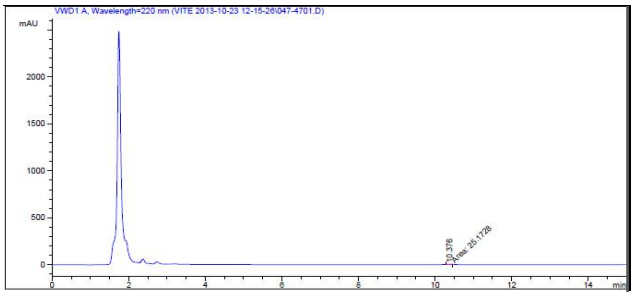


Fig. 6: Chloroform extract containsVit.E

Table 4: Quantitative analysis of Vitamin E in all the plant extracts using y = mx + C

Sr. No.	Sample name	Extract	Retention time in minutes	Response	Concentration in µg/mL
STD	Standard Sample dl a-tocopherol acetate	Methanolic solution	10.575	287.717	10
STD	Standard Sample dl a-tocopherol acetate	Methanolic solution	10.674	761.253	25
STD	Standard Sample dl a-tocopherol acetate	Methanolic solution	10.738	1594.99	50
STD	Standard Sample dl a-tocopherol acetate	Methanolic solution	10.719	3023.3	100
1	*Euphorbia fusiformis*	Chloroform	10.362	5.95727	0.018

The quantitative estimation of lipophilic vitamin E in tuber extracts of *Euphorbia fusiformis*is depicted in table 4. The chloroformextracts showed (0.018 µg/mL) concentration of vitamin E (Fig.6).

Vitamin C

The hydrophilic vitamin C(L-Ascorbic acid or L ascorbate) an essential nutrient for humans and other animal species, has been detected in aqueous tuber extract of *Euphorbia fusiformis*.The vitamin C and bête-carotene, which is the precursor for vitamin A, are important for absorption of some nutrients and eye vision. Therefore, the edible orchids can provide vitamin C for the people (elderly and refugees) who live in the areas where fresh fruits are limited (Kasulo, *et al.,* 2009).

Anti-scavenging activity

Many reports are available on the protective effects of natural anti-oxidants against oxidative stress related disorders like ageing, degenerative diseases and cancer (Cozzi, *et al.,* 1997). The phenolic compounds may have a direct contribution in anti-oxidant activity (Bidchol, *et al*., 2011).

The focal point of our present study was to explore the ethano-pharmacological significance of genobiotic medicinal plants of Euphorbiaceae family possessing diversified chemical nature. The phytochemical screening of the crude plant extracts showed the positive reaction for alkaloids, flavonoids, phenolic compounds, saponins, glycosides, carbohydrates, vitamin C, vitamin E and minerals.

The scavenging ability assayed is the ability of extracts to react rapidly with DPPH radicals and reduce most DPPH radical molecules. The anti-oxidant capacity of*Euphorbia fusiformis*tuber extracts was measured by DPPH anti-scavenging activity method and the results were expressed in Fig. 8. TheDPPH anti-scavenging activity of water extract (76.92%) and methanol extract (56.47%) were comparable. The water extract displayed significant anti-oxidant activity. These results might suggest higher medicinal suitability of alcoholic extracts in various anti-oxidant applications.

The observations depicted in Fig.8 suggested that the water extract displayed highest antioxidant activity. The results showed a positive correlation of Phenolic compounds, total flavanoids and vitamin E with anti-oxidant activity. On the other hand, a considerable DPPH radical scavenging activity was found in water extracts suggested the correlation of vitamin C and anti-oxidant activity. Decline in activity in chloroform extracts was recorded, which conferred the presence of phenols and vitamin E at minimal concentration in the extract. However, acetone extract exhibited 16.51% anti-oxidant activity attributable to presence of phenols.

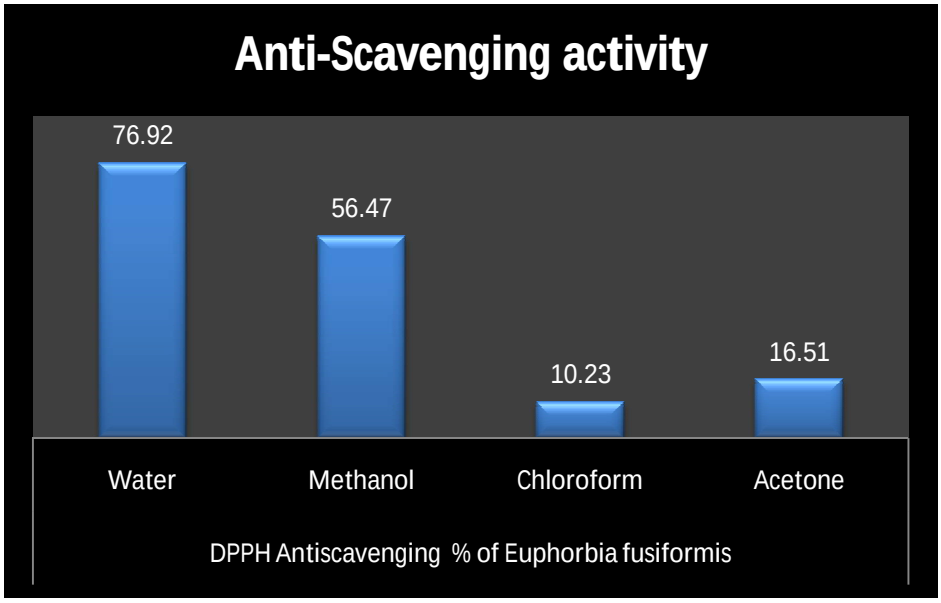


Fig. 7Anti-scavenging (DPPH) Activity oftuber extracts of*Euphorbiafusiformis*

***In vitro* anti-microbial activity**

All extracts were screened *in vitro* for their anti-microbial activities against clinically isolated bacterial and fungal strains such as *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonellatyphi, Escherichia coli* and *Aspergillus terreus.*

It was found that the methanolic and aqueous extracts of the species displayed moderate activity against all the microbial strains. Whereas, the microbial strains were found to be highly resistant to acetone, chloroform and IPA extracts.

The anti-microbial screening was carried out by disc diffusion method in respective solvents. The zone of inhibition was expressed in mm and compared with control solvents. The results are displayed in Table 5.

Flavonoids analysis by HPTLC

Flavonoids, the most important and most diverse natural phenolics (Agarwal, 1989) have diverse chemical and biological activities including radical scavenging properties. Fig.9 and 10 show the HPTLC profiles of aqueous, acetone, chloroform and methanolic tuber extracts of *Euohorbiafusiformis.* In HPTLC techniques, the flavonoids from methanolic extracts were determined by using solvent systemethyl acetate, acetic acid, formic acid and water (100:11:11:27) as a gradient mobile phase.

In the chromatogram of *Euphorbia fusiformis* using water extract, a total 8 peaks were obtained at 254nm and 366 nm as showed in the Plate - 19. The R_f values were compared with standards and literature precedence. All values were good in conformity. The concentration of Saponanin (R_f = 0.21) was found abundantly, whereas the flavonoids like Rutin (R_f = 0.42), Diosmin (R_f = 0.32), Epigenin (R_f = 0.49), Hesperidin (R_f = 0.61) were observed with moderate concentration in aqueous extract. A spot of phenolic acids like Caffeic acid (R_f = 0.79) was observed. The concentrations were confirmed by area under the peaks. The spots were analysed under UV light 245 nm after derivatization. Some unidentified flavonoid-glycosides were found present possessing low R_f values suggested that they have highest polarity. The TLC plate was screened under UV light at 366 nm after derivatization. At 366 nm, additional spot of Catechin (R_f = 0.96) was observed with moderate concentration. The other spots of flavonoids identified at 254nm were also located under 366nm.

The chromatogram of acetone plant extract was investigated at 254nm and 366 nm and a total 6 peaks were observed. Three unidentified flavonoids were observed whereas Hesperidin (R_f = 0.62), Catechin (R_f = 0.96), Astrangnlin (R_f = 0.67), Quercetin (R_f = 0.98) were identified at UV light at 254nm and 366 nm.

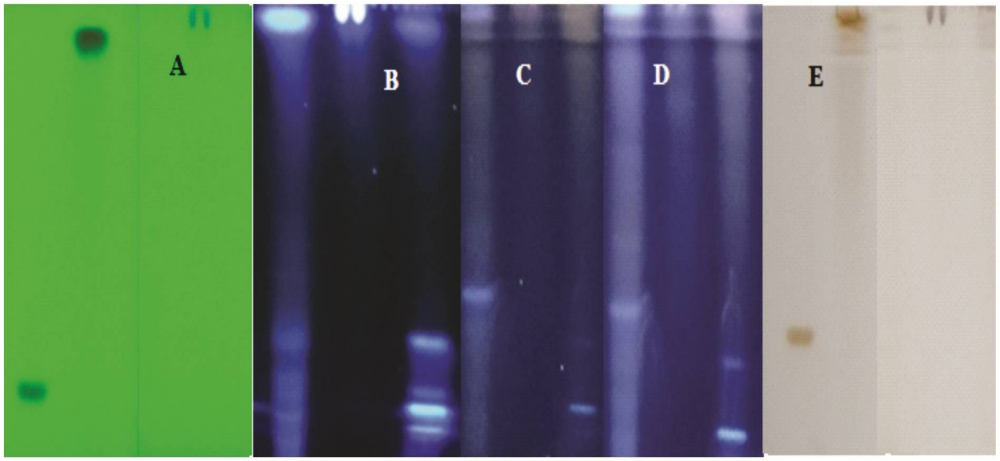
Five peaks were observed in the chromatogram of chloroform plant extract. Among five peaks, three peaks were unidentified and spots of Hesperidin (R_f = 0.58) and Quercetin (R_f = 0.98) were also located in the chromatogram. Additional two spots of Saponanin (R_f = 0.17) and Epigenin (R_f = 0.52) were located under UV light at 366 nm.

In methanolic plant extract, the chromatogram displayed 10 peaks, two remained unidentified, while 8 were identified as flavonoids and phenolic acids. Saponanin (R_f = 0.22), Diosmin (R_f = 0.31), Luteolin (R_f = 0.35), Rutin (R_f = 0.45), Hesperidin (R_f = 0.54), Astrangalin (R_f= 0.66), Phenolic acid (R_f = 0.73), Kaempferol (R_f = 0.82) were identified under UV light at 254nm. Two additional spots of Isoquericitrin (R_f = 0.53) and Catechin (R_f = 0.97) were seen. The results are depicted in Table-6 and Figure-8.

Table 5: Anti-bacterial and anti-fungal activities of *Euphorbia fusiformis*extracts

Sr. No.	Extract	*Salmonella typhi*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Escherichia coli*	*Aspergillus terreus*
1	Acetone	+	+	+	+	+
2	Chloroform	+	+	+	+	+
3	IPA	+	+	+	+	+
4	Methanol	++	++	++	++	++
5	Water	++	++	++	++	++

+ = 6-7 mm; ++ = 8-10 mm



A. HPTLC profile of the Water, Methanolic, Chloroform and Acetone extracts of *Euphorbia fusiformis*. Under UV254 BD.
B. HPTLC profile of the Water, Methanolic, Chloroform and Acetone extracts of *Euphorbia fusiformis*. Under UV366 BD.
C. HPTLC profile of the Water, Methanolic, Chloroform and Acetone extracts of *Euphorbia fusiformis*. Under UV254 AD.
D. HPTLC profile of the Water, Methanolic, Chloroform and Acetone extracts of *Euphorbia fusiformis*. Under UV366 AD.
E. HPTLC profile of the Water, Methanolic, Chloroform and Acetone extracts of *Euphorbia fusiformis*. Under Visible light AD.

Fig. 8: HPTLC Chemical Profiling of Flavonoids in tuber extracts of *Euphorbia fusiformis*

Table 6: Chemical profiling of tuber extracts of *Euphorbia fusiformis* under 254nm and 366nm after derivatization (AD)

Plant extract	254 nm AD				366 nm AD			
	R_f Value	Height (mm)	Area (AU)	Assigned substances	R_f Value	Height (mm)	Area (AU)	Assigned substances
BX-1-W	0.02	288.0	3323.0	Unknown	0.02	421.4	10858.4	Unknown
	0.02	413.3	10527.6	Unknown	0.21	15.3	33296.7	Saponanin
	0.21	14.9	31005.8	Saponanin	0.33	2.2	135.3	Diosmin
	0.32	4.3	185.7	Diosmin	0.42	38.1	1385.9	Rutin
	0.42	38.7	1670.1	Rutin	0.49	1.0	1524.8	Epigenin
	0.49	1.3	1405.2	Epigenin	0.62	5.5	1656.2	Hesperidin
	0.61	15.5	1777.6	Hesperidin	0.81	0.5	1480.7	Caffeic acid
	0.79	6.7	626.1	Caffeic acid	0.96	26.4	1335.7	Catechin
BX-1-A	0.04	14.3	360.3	Unknown	0.04	16.7	515.6	Unknown
	0.14	5.0	863.5	Unknown	0.14	4.7	1024.4	Unknown
	0.62	17.9	530.7	Hesperidin	0.63	16.7	556.9	Hesperidin
	0.67	4.0	212.0	Astrangnlin	0.67	5.2	199.5	Astrangnlin
	0.96	15.7	202.0	Catechin	0.96	18.2	278.2	Catechin
	0.98	8.9	215.0	Quercetin	0.98	9.9	226.7	Quercetin
BX-1-C	0.05	4.3	17282.6	Unknown	0.05	3.3	16920.2	Unknown
	0.10	8.3	159.0	Unknown	0.10	12.4	180.9	Unknown
	0.17	1.0	208.5	Unknown	0.16	5.2	668.7	Unknown
	0.58	30.1	741.2	Hesperidin	0.52	16.9	668.7	Epigenin
	0.98	42.3	2029	Quercetin	0.57	27.2	711.1	Hesperidin
					0.98	26.0	1191.6	Quercetin
BX-1-M	0.16	37.0	15962.1	Unknown	0.17	38.2	24333.5	Saponanin
	0.22	41.8	1379.7	Saponanin	0.22	43.5	1501.7	Unknown
	0.26	29.4	777.7	Unknown	0.26	30.1	785.5	Unknown
	0.31	36.2	1231.1	Diosmin	0.31	34.5	1200.1	Diosmin
	0.35	32.1	1346.4	Luteolin	0.35	33.4	1308.8	Luteolin
	0.45	55.5	2165.9	Rutin	0.53	37.5	4753.4	Isoquericitrin
	0.54	34.6	2699.1	Hesperidin	0.65	42.2	4483.6	Astrangalin
	0.66	37.1	4663.6	Astrangalin	0.73	28.7	964.7	Phenolic Acid
	0.73	23.2	781.6	Phenolic Acid	0.82	18.3	1694	Kaempferol
	0.82	14.4	1394.5	Kaempferol	0.97	28.5	899.2	Catechin

W – Water , A – Acetone , C – Chloroform , M – Methanol and BX-1 – Code for *Euphoribia fusiformis*

M: Methanol, C: Chloroform, A: Acetone and W: Water

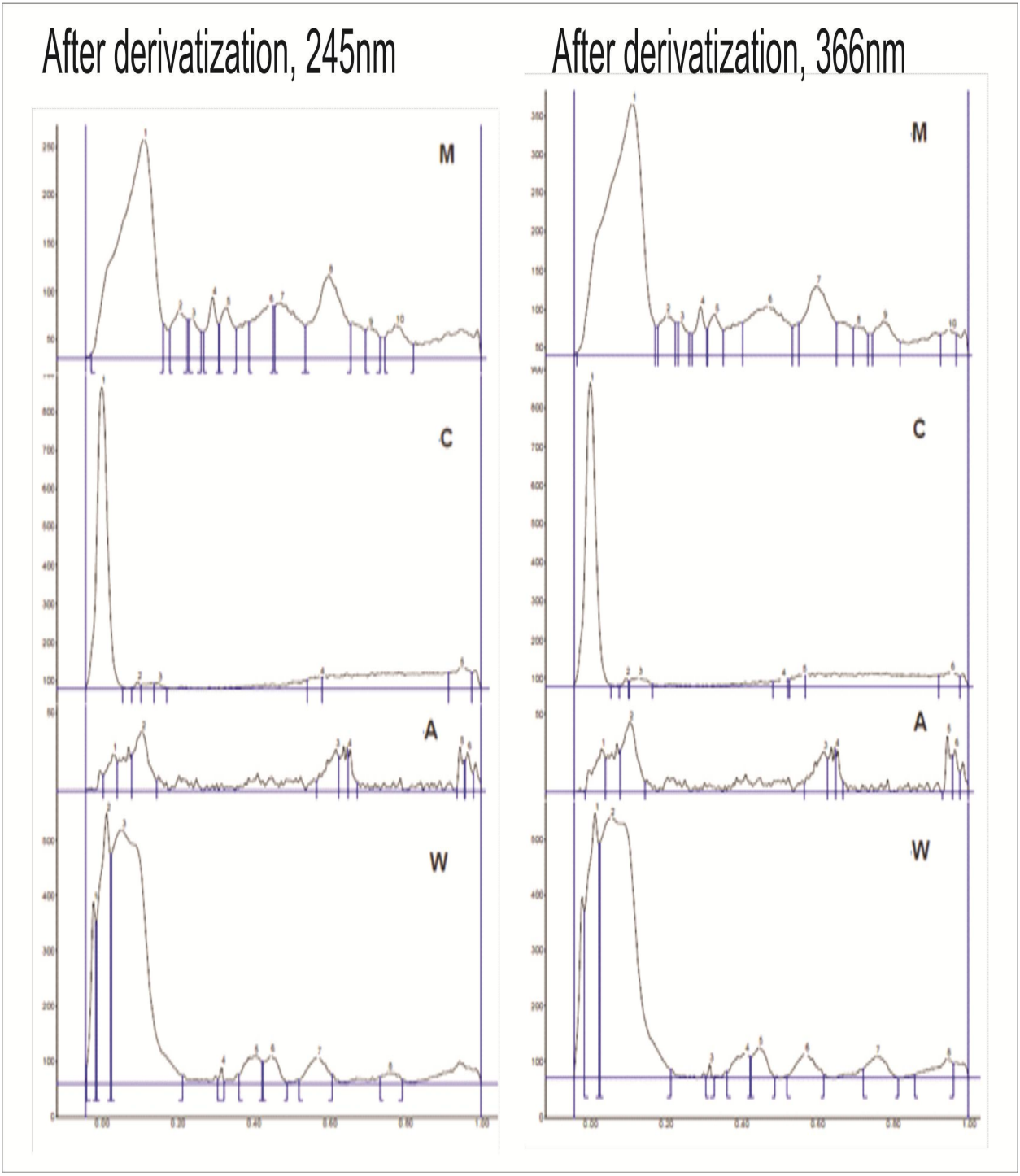
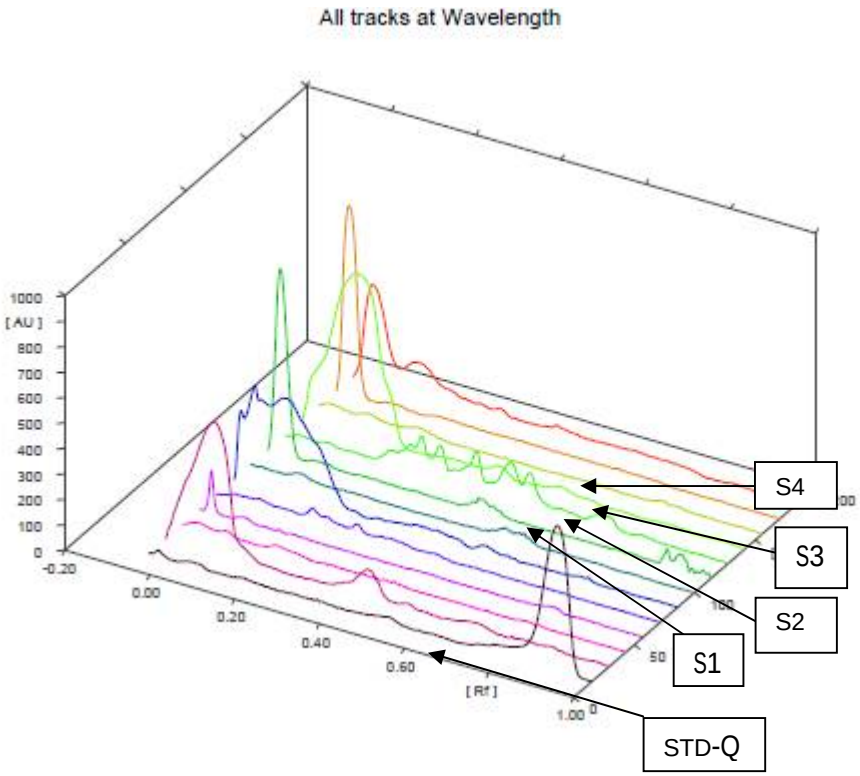


Fig. 9: HPTLC peaks at 254 nm and 366 nm after derivatization of tuber extracts of *Euphorbia fusiformis*



All peaks at 254 AD (Q-STD Quercetin, S1-Water,S2-Acetone,S3-Chloroform and S4-Methanol)
Fig. 10: HPTLC peaks of standard and tuber extracts of *Euphorbia fusiformis*

CONCLUSION

*Euphorbia*species have an ancient history of the multiple indigenous uses and is one of the most highly commercialized indigenous traditional medicines from India. Investigations of the phytochemicals and their biological activity have provided scientific support for many of its traditional uses. An improved RP-HPLC-UV-method has successfully applied for determination of dl α-tocopherol acetate in organic extracts of *Euphorbia fusiformis*. Similarly the results obtained from phytochemical analysis illustrated the occurrences of various micronutrients i.e.carbohydrates, vitamin C, vitamin E, flavonoids, phenols, glycosides, saponins and minerals i.e. Zn, Cu, Mn, Fe. The present findings for microelements suggested that their contents are responsible for significant anti-oxidant activity in all extracts.The quantitative estimation of vitamin E also showed its role as a natural anti-oxidant.The antimicrobial activity of all the extracts did not show promising results against the clinically isolated microbial strains. The detection of flavonoids by HPTLC also revels strong antioxidant activity in all the extracts. The structural characterisations (FTIR, NMR studies) of isolated flavonoids from various extracts of *Euphorbia fusiformis* are in progress.There is an urgent need of conservation and reintroduction of such medicinally important geophyte which is on verge of extinction.

ACKNOWLEDGEMENTS

The authors sincerely acknowledge the valuable support provided by Institute of Forensic Science, Institute of Science, Mumbai, Nagpur and Aurangabad; Lab India, Mumbai for ICP mineral analysis, THINQ Pharma Inc., Nilawar Laboratory, Wadhhamna, Nagpur for antioxidant activity.

REFERENCES

1. Agarwal PK.(ed) 1989. Carbon 13 NMR of flavonoids. Elsevier, Amsterdam.
2. Abidi SL.Chromatographic analysis of tocol-derivedlipid antioxidants. JChromatogr A.2000;881, 197-216.
3. Anusuya N, Raju K and ManianS. Hepatoprotective and toxicological assessment of an ethnomedicinal plant

*Euphorbia fusiformis*Buch.-Ham.exD.Don. JEtanopharmacol.2010;127(2):463-7.
4. AshaS, Deevika Band MohamadSadiqA.Euphorbia hirta Linn-a review on traditional uses, phytochemistry and pharmacology. World Journal of Pharmaceutical Research. 2014;3(4):180-205.
5. Banso Aand Adeyemo SO.Evalution of antibacterial Properties of tannins isolated from *Dichrostachyescinerea*. AfrBiotechnol.2007;6:1785-7.
6. Bidchol AM, Wilfred A, Abhijna P and Harish R.Free radical scavenging activ-ity of aqueous and ethanolic extract of *Brassica oleracea* L. var. italica. Food BioprocessTech.2011;4 (7):1137–1143.
7. Blatter E and McCann C. Revision of the flora of the Bombay Presidency, Part 15: Araceae. J of the Bombay Natural History Society. 1931;35:13 - 31.
8. Brand WilliumW,Cuveliear ME and Berset C. Use of free radical method to evaluate antioxidant activity.LebensonWissTechnol.1995;28:25-30.
9. British Nutrition Foudation.Energy and Nutrients. 1995.
10. Britto SJ, Soosairaj S, Natarajan D, Nagamurugan N and Ravipaul S. *Euphorbia fusiformis*Buch.-Ham. Ex.D. Don (Euphorbiaceae): A new record for Tamil Nadu. J of Economic and Taxonomic Botany.2002;26:467-471.
11. Cooke T. Araceae. *In:*TheFloraof the Presidency of Bombay (Taylor and Francis, London, U. K.).1906;2: 816–831.
12. Cozzi R, Ricordy R, Aglitti, T, Gatta, V, Petricone Pand De Salvia R. Ascor-bic acid and b-carotene as modulators of oxidative damage. Carcinogenesis.1997;18:223-228.
13. Duthie JF. Floraof the upper gangetic plain and of the adjacent Siwalik and Sub-Himalayan tracts. Government Printing Press, Calcutta, India.
14. Evans F and Taylors. 1983. Pro-infammatory, tumor promoting and anti tumorditerpene of the plant families Euphorbiaceae and Thymeleaceae. In: Herz W, Grisebach H, Kirby G. (Eds.), progress in the chemistry of organic Natural products, 44. Springer verlag,Neyork, pp. 1-99.
15. Gamble JS. Floraof the Presidency of Madras, I-III, Sri Giuranga Press Pvt. Ltd., Culcutta, India. 1957.
16. Harbone JB. Methods of extraction and isolation. *In:* Phytochemical methods,Chapman and Hall, London. 1998:60-66.
17. Hooker JD. Flora of British India,V: 584. Reeve and Co, London. 1887.
18. Kamal-Eldin A, Gorgen S, Pettersson J and LampiAM.Normal-phase high-performance liquid chromatography of tocopherols and tocotrienols - comparison of different chromatographic columns. JChromatogr A.2000;881**:**217-227.
19. Kasulo V, Mwabumba L and Munthal C. A review of edible orchids in Malawi. JHorti For. 2009;1(7):133-139.
20. Khandelwal KR. Prcticalpharmacognosy techniques and experiments. 2nd ed., Niraliprakashan, Pune. 2000.
21. Kingston HM and Jassie Lois B. Introduction to Microwave Sample Preparation: Theory and Practice, ACS Professional Reference Book Series, American Chemical Society, 1988.
22. Lee R. McDowell, Nancy Wilkinson, Rachel Madison and Tara Felix.Florida Ruminant Nutrition Symposium • Best Western Gateway Grand • Gainesville, FL, 2007;January,30-31.
23. McDowell LR. Vitamins in Animal and Human Nutrition, 2 nd ed., Iowa State university Press, Ames, IA. 2000.
24. Natarajan D, Britto SJ, Srinivasan K, Nagamurugan N, Mohanasundari Cand Perumal G. Anti-bacterial activity of Euphorbia fusiformis - A rare medicinal herb. J of Ethno Pharmacol. 2005;102: 123-126.
25. Prakash A and Singh KK. Use of medicinal plants by certain tribal people in North India. J of Tropical Medicinal Plants.2001;2: 225-229.
26. Raju K, Natarajan S, Karuppusamy S and Manian S. New ethno botanical report on Euphorbia fusiformis from Chitteri Hills of Tamil Nadu, India. J of Swamy Botanical Club. 2004;21: 79-80.
27. Ruperez FJ, Martin D, Herrera E and Barbas C. Chromatographic analysis of alpha-tocopherol and related compounds in various matrices. JChromatogr *A*.2001;935, 45-69.
28. Singh SB and Thakur RS. Aliphatic asters from *Costus specious* seeds.Ind J Pharm Sci. 1984:150-151.

29. Singla A and Pathak K. Phyto constituents of Euphorbia species.Fitoterapia. 1990;61:483-516.
30. Shah GL. Flora of Gujarat State,I and II.UniversityPress,Sardar Patel University,VallabhVidhyanagari, Gujrat, India, 1978.
31. Soumen A. A revision of geophytic Euphorbia species from India. Euphorbia World.2010;6 N(1) :19-21.
32. Swarnkar S and Katewa S. Ethnobotanical observation on tuberous plants from tribal area of Rajasthan (India). Ethnobotanical Leaflets.2008;12:647-66.