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Antioxidant and antimicrobial investigations of *Elaeocarpus tectorius* (Lour.) Poir. fruits against urinary tract infection pathogens.

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ABSTRACT

Plants are the natural honorarium used for primary health care and people rely upon herbal medications for the prevention and cure of numerous diseases that are caused by infectious pathogens. The extracts of *Elaeocarpus tectorius* fruits were successively prepared using five solvents (Petroleum ether, Dichloromethane, Ethyl acetate, Methanol and water) varying in their polarity ranges. The fruits of *E. tectorius* were screened for phytoconstituents such as alkaloids, flavonoids, terpenoids, steroids, glycosides that are endowed for the free radical scavenging activity. Fruit extracts showed significant amount of phenolics in ethyl acetate extract (302.33 ± 46.06 mg GAE/ g extract), tannins in methanol extract (60.53 ± 2.66 mg GAE/ g extract) and flavonoids in water extract (239.30 ± 9.79 mg RE/ g extract). Ethyl acetate extract was efficient showing maximum antioxidant activity with the lowest IC_{50} value in diphenyl-1-picrylhydrazyl radical-scavenging assay (20 µg/mL), ferric reducing/antioxidant power assay (3203.92 ± 413.62 mM (Fe(II)E/ mg extract) and phosphomolybdenum assay (881.00 ± 28.51 mg AAE/ g extract). Plants tend to express antimicrobial activity which are therefore twisting the use of harmful synthetic drugs and formulations in the recent years. In the antimicrobial evaluation the ethyl acetate extract was highly effective in Agar well diffusion assay followed by Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/ Fungicidal Concentration (MBC/ MFC) analysis against selective Urinary tract infection (UTI) pathogens (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Candida albicans*). Thus its more apparent that *E. tectorius* fruits show efficient activities and can be recommended as a herbal drug formulation for treating UTI.

1. Introduction

Under oxidative stress conditions the free radicals generated as by-products in living organisms through the metabolic process play a major role in the onset of pathophysiological disorders such as cancer, genotoxicity diseases, diabetes mellitus, early aging and stress related diseases. The diseases caused by multidrug-resistant (MDR) microbial pathogens create major problems in treating diseases even though there is progress achieved in human health care. Currently a number of synthetic chemical substances have been used as a drug to prevent disorders from free radicals and diseases from pathogenic microbes, but a lot of these synthetic drugs are nonspecific and fail to alleviate the diseases or disorders completely (Nurullaev, 2004; Orrett and Davis, 2006). The increasing failures and side effects associated with antibiotics have collectively made a requirement to look for new and effective drugs. During the past two decades, there has been increasing involvement in new broad spectrum drugs from plants as alternatives to synthetic drugs. The worldwide acceptance of herbal medicine through the screening of active compounds in plant extracts serve as a potential source of new antibiotic prototypes (Afolayan, 2003). Contrary to the synthetic drugs that possess serious side effects such as neurotoxicity, nephrotoxicity and hepatotoxicity, researchers give attention to natural antioxidants and antimicrobial agents from plant origin to develop safer drugs (Verma, 2017).

Urinary tract infections are the most common disease among mankind. Women are considered to be in more risk and prone to infections than men, as the incidence of UTI is greater and vulnerable in women compared to men (Salvatore et al., 2011). Clinical signs and symptoms consistent with uncomplicated UTI are e.g. Urethritis, dysuria, pyuria, frequency, back pain etc. However, *E. coli* being the highly frequent uropathogen may soar through the ureters to the kidneys pathway and can be even capable of causing more severe infections such as pyelonephritis and cause high morbidity rate. Infections in urinary tract may even lead to development of rashes and wounds. Antibiotics that are

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taken in oral form such as trimethoprim, losporins, nitrofurantoin or a fluoroquinolone helps in short time remedial action (Grigoryan et al., 2014). Due to the widespread use of these medications for UTI, a condition of resistance has developed (Raju and Twari, 2001). Plants are being used by the people for primary health care and to treat infectious diseases. The indigenous knowledge in use of traditional system of medicine is recognized as the habitual treatment for several maladies and ailments (Rios and Recio, 2005).

E. tectorius (Lour.) Poir. syn *E. oblongus* belonging to the Elaeocarpaceae family is one among the traditional tree species that bear edible fruits and is consumed by the tribal people of the Western Ghats. Reports confirmed that most of the *Elaeocarpus* species have exhibited anti-inflammatory, anti-microbial, anti-anxiety, analgesic, anti-depressant, anti-asthmatic, anti-diabetic, anti-ulcerogenic, anti-tumor, cytotoxic, sedative, hypnotic, tranquillizing, anti-convulsive, anti-epileptic and anti-hypertensive properties through the pharmacological investigations (Dadhich et al., 2013). They also have many biological active molecules such as indolizilidine alkaloids, triterpenes, tannins, grandisines, rudrakine fatty acids, ellagic acid derivatives, cytotoxic compounds flavonoids and quercitin that confirm the presence of phytochemicals, polyphenols and antioxidants (Muthuswamya, 2014). The fruits are used in the treatment of rheumatism, pneumonia, ulcers, piles and leprosy (Dadhich et al., 2013). With this backdrop information the present study was intended and carried out to screen the phytochemical compounds, determine the total polyphenolics content, antioxidant and antimicrobial activity of the fruit extracts of *E. tectorius*. And this is the first report of antimicrobial activity of the plant against selected pathogens.

2. Materials and methods

2.1. Collection and preparation of plant material

The fruits of *E. tectorius* were collected during the month of October 2017 from Coonoor, The Nilgiris district, Tamilnadu, India. The plant was identified by its taxonomic characters comparing with the voucher specimen at the Madras Herbarium (MH) of Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamil Nadu (Reference no. BSI/SRC/5/23/2018/Tech./2853). Freshly collected fruits were cleaned to remove the external adhering dust; the skin was pealed excluding the seed and then dried under shade. The dried sample was then finely powdered and used for further analysis.

2.1.1. Preparation of extracts

The powdered fruit material was subjected to extraction by using the Soxhlet apparatus. The sample of about 100 g was successively extracted with petroleum ether, dichloromethane, ethyl acetate, methanol and water at temperature varying at the range of 20–40 °C. The fruit material was dried in hot air oven below 40 °C before adding the next solvent. The different solvent extracts were concentrated by using the rotary vacuum evaporator (Equitron Ev11-ABS.051) and then dried at room temperature. The amount of crude extracts recovered was weighed and the percentage of yield was calculated. Then the dried extract can be stored at −20 °C. The extract recovery in different solvents was expressed as percent of the dry matter. The amount of crude extracts recovered after successive extraction were weighed and the percentage of yield was calculated by the following formula, Extract recovery percent = [Amount of extract (g)/Amount of dried plant sample (g)] x 100.

2.2. Chemicals

Folin - Ciocalteu reagent, Sodium carbonate, PVPP (polyvinyl polypyrolidone), Sodium nitrite, Aluminium chloride, Sodium hydroxide, DPPH (2,2-Diphenyl-1-picryl-hydrazyl), Rutin, Gallic acid, TPTZ (2,4,6-tripyridyl-*s*-triazine), ferrous chloride, ferric chloride, Hydrochloric acid, Sulphuric acid, sodium phosphate, ammonium molybdate, Ascorbic acid, Mueller Hinton agar (MHA), Sabouraud Dextrose agar (SDA), Luria–Bertani broth, Sabouraud Dextrose broth, Standard antibiotics etc. were purchased from Hi-media (Mumbai), SRL (Mumbai) and Sigma Aldrich (USA). All the chemicals and solvents were used of analytical grade.

2.3. Preliminary phytochemical analysis

The preliminary qualitative phytochemical screening of the *E. tectorius* fruit powder was done to find out the different phytochemical constituents such as alkaloids, phenolic compounds, tannins, flavonoids, terpenoids, saponins, glycosides and steroids using standard methods (Harborne, 1998; Thangaraj, 2016).

2.3.1. Hager's test for alkaloids determination (Wagner et al., 1996)

About 50 mg solvent free powder was stirred with 5 mL of dilute hydrochloric acid and filtered. To the filtrate, 2 mL of Hager's reagent (aqueous solution of picric acid) was added. A yellow precipitate appears that indicates the presence of alkaloids.

2.3.2. Ferric chloride test for phenolics determination (Mace, 1963)

About 50 mg of the powder was dissolved in 5 mL of distilled water. To this, few drops of 5% neutral ferric chloride solution was added. Phenolic compounds were indicated by the presence of dark green colour.

2.3.3. Potassium hydroxide test for tannins determination (Williamson et al., 1996)

The fruit powder (500 mg) was added into 10 mL of freshly prepared 10% potassium hydroxide (KOH) in a beaker and shaken to dissolve. A dirty precipitate formation indicated the presence of tannins in the sample.

2.3.4. Alkaline reagent test for flavonoids determination (Raaman, 2006)

An aqueous solution of the powder was treated with 10% ammonium hydroxide solution. Appearance of bulky white precipitate indicated the presence of flavonoids.

2.3.5. Liebermann-Burchard reaction for terpenoids determination

About 50 mg of the powder was added to 1 mL of chloroform, was mixed and then added to acetic anhydride followed by concentrated sulphuric acid from the sides of the tubes. Appearance of red and bluish green colour indicated the presence of steroids and triterpenoids.

2.3.6. Frothing test for saponins determination (Kokate, 1999)

The fruit powder (50 mg) was diluted with distilled water and made up to 10 mL. The suspension was shaken in a graduated cylinder for 15 min; increase in layer of foam indicated the presence of saponins.

2.3.7. Borntrager's test for glycosides determination

About 50 mg of powder was hydrolyzed with concentrated hydrochloric acid for 2 h on water bath and filtered. To 2 mL of filtrate, 3 mL of chloroform was added and shaken. The chloroform layer was separated and 10% ammonia solution was added to it. Formation of pink colour indicated the presence of glycosides.

2.3.8. Salkowski test for steroids determination

2 mL of chloroform and 1 mL concentrated sulphuric acid were added to 10 drops of the powder mixed with isopropyl alcohol, slowly until double phase formation. The presence of a dish-brown color in the middle layer marks the presence of steroidal ring.

2.4. Determination of total phenolics

The quantitative estimation of phenolics in the *E. tectorius* fruit

extracts were determined based on the standardized method (Siddhuraju and Becker, 2003). About 0.5 mL of 1N Folin-Ciocalteu reagent and 2.5 mL of 20% sodium carbonate solution were added sequentially in each tube containing different solvent extracts, followed by 40 min dark incubation and the absorbance was recorded at 725 nm against blank for the estimation of phenolics. The results were based on the calibration curve: $y = 0.025x - 0.056$, $R^2 = 0.998$ where x was the absorbance and y was the gallic acid equivalents (mg/g) and were expressed in terms of milligrams gallic acid equivalents (GAE) per gram of extract.

2.5. Determination of total tannins

Tannin estimation (Siddhuraju and Manian, 2007) was done by treating the *E. tectorius* fruit extracts with polyvinyl polypyrolidone (PVPP). 500 µL distilled water and 500 µL of the sample extracts were added to 100 mg of PVPP. The content was then vortexed and kept in an eppendorf tube at 4 °C for 10–15 min. Then the sample were centrifuged at 4000 rpm for 10 min at room temperature and the supernatant was collected that consists of only simple phenolics, whereas the tannins would have been in a precipitated state along with PVPP. Thus the phenolic content of the supernatant was measured and expressed as the content of non-tannin phenolics. The amount of tannins was calculated by subtracting the non-tannin phenolics from total phenolics. The results for tannins are expressed in gallic acid equivalents.

2.6. Determination of total flavonoids

Total flavonoid in the extracts is estimated by the general procedure (Zhishen et al., 1999). To each 300 µL of *E. tectorius* fruit extracts 2 mL of distilled water was added followed by 150 µL of $NaNO_2$. The contents of the tubes were subjected to incubation for 6 min at room temperature. After incubation 150 µL of $AlCl_3$ (10%) was added and incubated again for 6 min at room temperature. Then 2 mL of 4% NaOH was added, vortexed well and kept at room temperature for another 15 min. The absorbance of pink colour thereby developed was read spectrophotometrically at 510 nm. The results were based on the calibration curve: $y = 0.002\ x - 0.008$, $R^2 = 0.996$ where x was the absorbance and y was the rutin equivalents (mg/g) and the results were expressed in terms of milligrams rutin equivalents per gram of extract.

2.7. In vitro antioxidant assays

2.7.1. DPPH radical scavenging activity

The radical scavenging activity of extracts was determined by the standardized method of DPPH radical scavenging activity (Blois, 1958). A methanol solution of the sample extracts at various concentrations was added to 5 mL of 0.1 mM methanolic solution of DPPH and allowed to stand for 20 min at 27 °C. The absorbance of the solution was read at 517 nm using spectrophotometer. Methanol was served as blank and solution without fruit extract served as the negative control. The mixture of methanol, DPPH and standard rutin served as the positive control. The radical scavenging ability of the extract is expressed by IC_{50} of the extracts.

2.7.2. Ferric reducing antioxidant power (FRAP)

The antioxidant capacities of extracts of samples were estimated according to the standard method described by Pulido et al. (2000). The antioxidant potential of various extracts of *E. tectorius* fruits was estimated to analyse their ability to reduce TPTZ–Fe (III) complex to TPTZ–Fe (II) complex. About 100 µL of plant sample was added to freshly prepared 900 µL of FRAP reagent [2.5 mL of 20 mM TPTZ (2,4,6-tripyridyl-*s*-triazine) solution in 40 mM HCl and 2.5 mL of 20 mM $FeCl_3 \cdot 6H_2O$ and 25 mL of 0.3 M acetate buffer with pH 3.6] and was incubated at 37 °C in water bath for about 30 min. Methanol in the place of sample along with the FRAP reagent served as the blank. At the end of incubation, the absorbance were read immediately at 593 nm. The FRAP value was expressed as mM Fe(II) equivalents/ mg extract.

2.7.3. Phosphomolybdenum activity assay

The antioxidant activity of samples was evaluated by the phosphomolybdenum method (Prieto et al., 1999). Fruit extracts along with 1 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated in a water bath at 95 °C for 90 min. Then it was let to cool to room temperature, the absorbance of the mixture was measured at 765 nm against a blank. The results were reported in ascorbic acid equivalents (AAE)/ g extract.

2.8. Antimicrobial activity

2.8.1. Test pathogens

Pure cultures of *Escherichia coli* (MTCC- 433), *Staphylococcus aureus* (MTCC- 737), *Pseudomonas aeruginosa* (MTCC- 741) and *Candida albicans* (MTCC- 227) were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh. The pure bacterial cultures were maintained on nutrient agar medium and fungal culture on SDA medium.

2.8.2. Agar well diffusion method

The antimicrobial activity of *E. tectorius* fruit extracts against selected UTI pathogens was determined by the modified agar well diffusion method (Perez, 1990). Broth cultures of bacteria were swabbed in plates with MHA media and *Candida* (yeast fungal pathogen) culture in SDA medium using sterile cotton swabs. Wells were made by the help of a sterilized cork borer (6 mm Hi-Media). The extract samples in different solvents were dissolved in 4% DMSO. Diluted samples (100 µL of 10 mg/mL concentration) were filled in the wells and then the plates were aerobically incubated at 37 °C for 18-24 h for bacterial pathogens and 28 °C for 48 h fungal pathogens respectively. Ampicillin-15 µg (bacteria), Miconazole-30 µg (*Candida*) and 4% DMSO were considered as the positive and negative controls. After required period of incubation the zones of inhibition (ZOI) (mm) of each sample were measured and the activity index was also calculated. The reading were taken in triplicate basis and average values were recorded. The whole assay was done under *in vitro* conditions.

2.8.3. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/ Fungicidal Concentration (MBC/ MFC) of E. tectorius fruit extracts

MICs were carried out for the serial extract of *E. tectorius* fruit (That show ≥ 8 mm diameter growth ZOI in well diffusion method) in broth dilution method. The 5 mg/mL concentration of extract was serially diluted in 900 µL sterile broth (Bacteria- MHA broth; *Candida*- SDA broth). Then all the broth were filled with 100 µL of the culture (1×10^5 CFU/mL) and one was kept as control for each test organisms, then all culture tubes were incubated at 37 °C. After incubation OD values were taken in spectrophotometer (Shimadzu UV-spectrophotometer, UV-1800) at 570 nm. From OD values the percentage of growth inhibition and MIC were calculated against test pathogens. The MBC/MFC was determined by sub culturing the test dilution used in MIC, after incubation, the culture pellets obtained from MIC test by centrifugation (Eppendorf centrifuge 5430 R) was suspended in 100 µL sterile broth and the total suspension swabbed onto the desired (Bacteria- MHA; *Candida*- SDA) plates and allowed to incubate for a further 24-48 h at 37 °C on to a fresh solid medium and incubated further for 24 h. The concentration of plant extract that completely killed the organisms was considered as MBC/MFC (Wayne, 2002, 2010).

2.9. Statistical analyses

The results were expressed as Mean ± SD. The data were

Table 1
Extract yield percentage of *E. tectorius* fruit extracts.

Plant material used	Solvents	Extract yield (%)
Fruit powder	Petroleum ether	0.70
	Dichloromethane	0.30
	Ethyl acetate	0.60
	Methanol	11.51
	Water	4.32

statistically analysed using SPSS version 17.0 by means of one way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) for total polyphenolics and antioxidant studies. Mean values were considered statistically significant when $p < 0.05$.

3. Results and discussion

3.1. Extract yield percentage

Extraction process was done using the soxhlet apparatus in a successive manner, which is considered as the traditional method used for decades (Kaufmann and Christen, 2002). The highest yield was found to be in the methonolic extract with about 11.51%, whereas the minimum yield was in dichloromethane extract with just 0.3% comparatively (Table 1). This shows that the polar solvents are capable of extracting the maximum amount of organic bioactive compounds from *E. tectorius* fruits. The difference in the extract yield mainly depends on the phytoconstituents, their nature of solubility, polarity of the solvents and temperature. Plant extracts are thus obtained with several bioactive compounds or phytochemicals with varied polarities.

3.2. Qualitative phytochemical screening

Positive results were shown for the presence of alkaloids, phenolic compounds, tannins, flavonoids, saponins, glycosides, terpenoids and steroids (Table 2). The behaviour of the fruit powder when reacted to chemical reagents shows the character uniqueness for detecting the phytoconstituents. Fruits are said to be one of the major nutritive parts plants. The active phytochemicals are associated to provide protection from serious cardiovascular diseases, cancer, diabetis, hypertension, cholesterol and all other medical conditions (Craig, 1997). They are the bioactive compounds that contribute in offering profound health benefits (Đilas et al., 2009).

Alkaloids are those which naturally occurs as chemical compounds in plant parts that often have pharmacological effects. Phenolic compounds tend to show antioxidant properties as oxygen scavengers, peroxide decomposers, metal chelating agents, and free radical inhibitors; also possess anti-tumor, anti-bacterial, anti-viral anti-mutagenic and cardio protective properties (van Acker et al., 1998). Tannins are known to be the high molecular weight phenolics that precipitate protein (Hagerman et al., 1998). Flavonoids are capable of scavenging oxygen-derived free radicals also possess anti-inflammatory, anti-allergic, anti-viral, and anti-carcinogenic properties (Middleton, 1998). Saponins are the bioactive compounds with both biological and pharmacological properties, that naturally occur in plants as triterpenes or steroid glycosides (Lacaille-Dubois and Wagner, 2000). Terpenoids which are plant based compounds have been used in the food traditionally; also act as a source primarily in pharmaceutical and chemical industries. They are recently been used in developing biofuel products as well (Tholl, 2015). Glycosides are the secondary plant metabolites under the polyphenolic group that commonly occur in plants with various anti-inflammatory properties (Wong et al., 2006). Plant Steroids have antibacterial properties; they possess many medicinal, pharmaceutical and agrochemical activities and are known to enhance the immune response as well (Epand et al., 2007; Patel and Savjani, 2015). The presence of these biologically active compounds in the fruit has several properties especially by fighting against pathogens they prove to possess antimicrobial ability, antioxidant capacity by scavenging the harmful free radicals, therapeutic potential and serve to be a better nutraceutical.

Table 2
Phytochemical screening of *E. tectorius* fruit powder.

Phytochemical constituents	Fruit
Alkaloids	+ + +
Phenolic compounds	+ +
Tannins	+
Flavonoids	+
Saponins	+ +
Glycosides	+
Terpenoids	+
Steroids	+ +

(+): Presence of chemical compound, (−): Absence of chemical compound; +: Low intensity of characteristic colour, + +: Medium intensity of characteristic colour; + + +: Higher intensity of characteristic colour.

3.3. Determination of total phenolics, flavonoid, and tannin contents

The quantitative phytochemical screening of total phenolics, flavonoid and tannin content was analysed in all the extracts of *E. tectorius* fruit (Table 3). From the analysis it's clear that the extracts of the middle and highly polar solvents show better results for the high polyphenolic contents. The ethyl acetate extract showed maximum value for the total phenolics (302.33 ± 46.06 mg GAE/g of extract). Tannins were comparatively high in the methanol extract (60.53 ± 2.66 mg GAE/g extract), based on the significance level it was found high in ethyl acetate (56.13 ± 6.46 mg GAE/g extract) and water extract (49.33 ± 4.54 mg GAE/g extract) as well; whereas the water extract of fruit revealed high amount of total flavonoid content (239.30 ± 9.79 mg RE/g of extract). However, the minimum amount of the polyphenolic contents was found to be in the petroleum ether extract which may be due to its non polar nature and inability to dissolve active biomolecules. The polyphenolic constituents possess several activities such as free radical scavenging, anti inflammatory, anti carcinogenic, antimutagenic, anti microbial etc. The substantial quantity of those compounds likely aids better for the antioxidative and antimicrobial potential.

Total polyphenolic content play a significant role in antioxidation as well as important biological function of the plant (Saravanan and Parimelazhagan, 2014). Flavonoids and tannins are the phenolic compounds that belong to the chief group of plant phenolics, they are free radical scavengers and are primary antioxidants (Muniyandi et al., 2017). Since these compounds were found to be present in the fruit extracts it might be responsible for the potent antioxidant capacity in scavenging the free radicals.

Table 3
Determination of total phenolics, tannins and flavonoids content in *E. tectorius* fruit extracts.

Fruit extracts	Total phenolics (mg GAE/g extract)	Total tannins (mg GAE/g extract)	Total flavonoids (mg RE/g extract)
Petroleum ether	38.33 ± 6.35^{c}	29.46 ± 6.07^{c}	21.110 ± 2.13^{e}
Dichloromethane	46.00 ± 4.00^{c}	35.60 ± 13.75^{bc}	159.02 ± 4.41^{b}
Ethyl acetate	302.33 ± 46.06^{a}	56.13 ± 6.46^{a}	89.160 ± 1.66^{c}
Methanol	192.46 ± 10.21^{b}	60.53 ± 2.66^{a}	50.830 ± 3.63^{d}
Water	175.40 ± 3.89^{b}	49.33 ± 4.54^{ab}	239.30 ± 9.79^{a}

Values are mean of triplicate determination (n = 3) ± standard deviation, GAE- Gallic Acid Equivalents, RE- Rutin Equivalents. The mean values were statistically significant at $p < 0.05$ where $^{a} > ^{b} > ^{c} > ^{d} > ^{e}$ in each column.

Table 4
Determination of DPPH scavenging activity, FRAP assay and Phosphomolybdenum assay of *E. tectorius* fruit extracts.

Fruit extracts	DPPH radical scavenging activity IC_{50} value (μg/mL)	FRAP assay (mM Fe(II)E/mg extract)	Phosphomolybdenum assay (mg AAE/g extract)
Petroleum ether	22.88[b]	102.25 ± 1.48[c]	93.33 ± 2.75[d]
Dichloromethane	28.54[c]	201.86 ± 23.20[c]	300.00 ± 6.61[c]
Ethyl acetate	20.00[a]	3203.92 ± 413.62[a]	881.00 ± 28.51[a]
Methanol	43.93[d]	1947.05 ± 90.55[b]	653.33 ± 132.80[b]
Water	51.85[e]	1837.25 ± 198.29[b]	734.33 ± 48.08[b]
Rutin (control)	10.83	4705.69 ± 27.66	312.22 ± 30.97

Values are mean of triplicate determination (n = 3) ± standard deviation, AAE- Ascorbic Acid Equivalents. Statistically significant at $p < 0.05$ where $^{a} > ^{b} > ^{c} > ^{d} > ^{e}$ in each column.

3.4. In vitro antioxidant assays

Antioxidants are the organic substances that are highly utilised through natural sources which is also a combination of complex phytocompounds. Due to extreme generation of oxidative stress by prooxidants, a condition is developed where the cellular molecules such as proteins, lipids, and nucleic acids suffer oxidative damages and may cause tissue disruption (Halliwell and Aruoma, 1991; Halliwell and Gutteridge, 2015). So the antioxidants found in the fruits of *E. tectorius* can play a vital role in stabilizing the free radicals. The antioxidant capacity of the fruit extracts were studied to find the ability of free radical scavenging property (Table 4). The DPPH scavenging activity was studied by calculating the IC_{50} values for all the solvent extracts. Based on the results it was found that the minimum IC_{50} value was in the ethyl acetate extract (20 μg/mL) that proved to show the higher ability to scavenge the DPPH radical. Similarly in FRAP and Phosphomolybdenum assay also the efficient free radical scavenging activity was considerably high in the ethyl acetate extract (3203.92 ± 413.62 mM Fe(II)E/mg extract), (881.00 ± 28.51 mg AAE/g extract) and showed higher significance. By this it is evident that the ethyl acetate solvent has solubilised the essential biomolecules from *E. tectorius* fruit sample which shows the presence of important antioxidants that are capable of stabilizing the unstable free radicals. The highly enhanced activity of ethyl acetate extract may be due to its polar nature. However, the lowest reducing ability was seen in dichloromethane and petroleum ether extracts which is due to its non polar nature and solubilizes mostly the volatile compounds alone.

DPPH radical scavenging activity is the convenient, easiest and fast method for screening antioxidants in plant extracts (Koleva et al., 2002). Reports show that peels, pulps and seeds of fruits show stronger antioxidant activities based on their FRAP values as the fruit possess relative amount of natural antioxidants (Guo et al., 2003). The phosphomolybdenum assay is based on the reduction of Mo(VI) to Mo(V) by antioxidant compounds that are generally found in *E. tectorius* fruit extracts and subsequently is indicated by the formation of a green phosphate Mo(V) compounds (Prieto et al., 1999). So the optimal health of human beings can be maintained by high fruit intake as the antioxidants in the fruits are concerned with reduced mortality, cardiovascular disorders and cancer (Lampe, 1999).

3.5. Antimicrobial activity

The antimicrobial activity was tested for all the extracts of *E. tectorius* fruit samples (Table 5) (Fig. 1). Four microbial infectious strains such as *E. coli, S. aureus, P. aeruginosa* and *C. albicans* which are responsible for UTI in a high rate were subjected for analyses. The antimicrobial potential of the extracts of *E. tectorius* fruit (Petroleum ether, Dichloromethane, Ethyl acetate, Methanol and water) was evaluated by agar well diffusion method using MHA medium and SDA medium. The extracts also exhibited variable degrees of antimicrobial activity for all the strains.

The diameter of the ZOI against each solvent extracts were read for each test pathogen that comprised a well size of 6 mm in diameter. The ethyl acetate extract show the maximum ZOI thus proving its antimicrobial potential. Though there were certain variations for bacterial and candidal test pathogens, the effective result was seen in ethyl acetate extract. The maximum antibacterial activity in ethyl acetate extract of fruit against *S. aureus* (ZOI - 14.00 ± 1.00 mm; MIC - 250 μg/mL; MBC - 400 μg/mL), *E. coli* (ZOI - 16.50 ± 0.50 mm; MIC - 200 μg/mL; MBC - 300 μg/mL); *P. aeruginosa* (ZOI - 14.00 ± 1.00 mm; MIC - 250 μg/mL; MBC - 350 μg/mL). Similarly *C. albicans* exhibited higher susceptibility against *E. tectorius* fruit extract with ZOI of 21.00 ± 2.00 mm; MIC - 150 μg/mL and MFC - 200 μg/mL respectively. However, there was insignificant antimicrobial activity in the petroleum ether and dichloromethane extracts which may be due to its non polar nature and inability of extracting bioactive compounds. From the results it is inferred that the antioxidant and antimicrobial activities correlates with each other. Hence proves the potency of ethyl acetate extract to be more efficient in inhibiting the UTI causing pathogens. The biocompounds of the fruit enables to counteract the growth of bacteria and fungi.

Plants are a source of natural compounds that act as antimicrobial drugs and anti-infection agents for the past few decades utilised as part of the habitual treatment of various maladies and ailments (Rios and Recio, 2005). The shift to natural drugs is because microorganisms have become more resistant to synthetic antibiotics. The use of bearberry (*Arctostaphylos uva-ursi*) and cranberry juice (*Vaccinium macrocarpon*) is highly preferable to treat urinary tract infections is reported in various manuals of phytotherapy and pharmacognosy, while many plants such as *Melaleuca alternifolia* (tea tree), *Melissa officinalis* (lemon balm) and *Allium sativum* (garlic) are described as antimicrobial agents against many pathogens in a broad spectral range (Heinrich et al., 2017). Therefore, the studies of *E. tectorius* fruits indeed have shown positive results against UTI pathogens for the prevention and cure of infections in the urinary tract. Plant based drugs does not cause any drastic side effects and are also considered to have enormous therapeutic potential in comparison with synthetic drugs and antibiotics.

4. Conclusion

E. tectorius is a wild tree species and is not known by most of the people for its edible fruits. The fruits are consumed by the tribal communities of Western Ghats for its medicinal values. Reports show that the fruits are the cheap source for the treatment of rheumatism, body pain, leprosy, pneumonia, ulcers, piles, and dropsy. The extracts of fruits, leaf, bark and stem revealed the high range of activities like antiviral, antibacterial, antifungal, positive effects on respiration, cardiovascular systems, central nervous system, diuretic and anti inflammatory properties that further brings the importance of the plant (Muthuswamya, 2014). Through our study the antioxidant, antibacterial and antifungal properties were analysed in the edible fruits of *E. tectorius* that exhibited its biological activity in the ethyl acetate, methanol and water extracts.

Therefore, from the present investigation it is evident that the fruit of *E. tectorius* contains the essential phytochemicals that act as antioxidants and also have specific antimicrobial activity against UTI. The

Table 5
Antimicrobial activity of *E. tectorius* fruit extracts.

Extracts	*S. aureus*			*E. coli*			*P. aeruginosa*			*C. albicans*		
	ZOI[a] (mm)	MIC (µg/mL)	MBC (µg/mL)	ZOI[a] (mm)	MIC (µg/mL)	MBC (µg/mL)	ZOI[a] (mm)	MIC (µg/mL)	MBC (µg/mL)	ZOI[a] (mm)	MIC (µg/mL)	MFC (µg/mL)
Petroleum ether	00.00 ± 0.00	/	/	00.00 ± 0.00	/	/	00.00 ± 0.00	/	/	00.00 ± 0.00	/	/
Dichloromethane	00.00 ± 0.00	/	/	00.00 ± 0.00	/	/	00.00 ± 0.00	/	/	00.00 ± 0.00	/	/
Ethyl acetate	14.00 ± 1.00	250	400	16.50 ± 0.50	200	300	14.00 ± 1.00	250	350	21.00 ± 2.00	150	200
Methanol	12.66 ± 0.57	300	450	15.00 ± 1.00	250	300	13.33 ± 1.25	350	450	16.66 ± 0.57	250	300
Water	11.33 ± 0.57	400	550	11.16 ± 0.76	400	550	10.83 ± 0.76	450	550	14.50 ± 0.50	250	350
4% DMSO	00.00 ± 0.00	/	/	00.00 ± 0.00	/	/	00.00 ± 0.00	/	/	00.00 ± 0.00	/	/
Ampicillin (15 µg)	16.60 ± 0.57	0.6	1.0	21.00 ± 1.00	0.8	1.0	20.00 ± 1.00	0.7	1.0	NA	NA	NA
Miconazole (30 µg)	NA	NA	NA	NA	NA	NA	NA	NA	NA	16.6 ± 3.78	1.00	1.50

[a] Data are presented in mean values (n = 3) ± Standard deviation at 1 mg concentration of extracts; ZOI- Zone of inhibition; MIC- Minimum inhibitory concentration; MBC- Minimum bactericidal concentration; MFC- Minimum fungicidal concentration; /- Not analysed; NA - Not applicable.

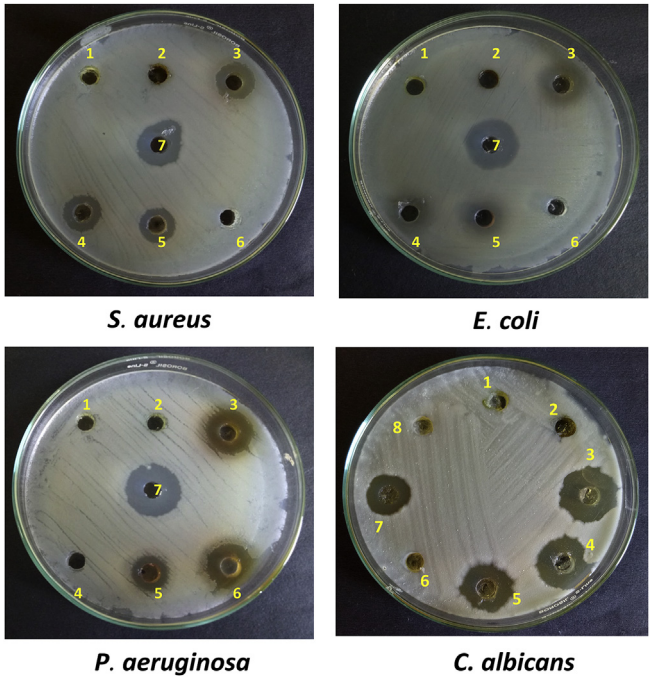

Fig. 1. Antimicrobial activity of *E. tectorius* fruit (1) Petroleum ether extract (2) Dichloromethane extract (3) Ethyl acetate extract (4) Methanol extract (5) Water extract (6) 4% DMSO control (7) Positive controls (Ampicillin/Miconazole) (8) Well control.

natural cure of infections and the healing of wounds caused by them can be done through plant formulations as well. The edible fruits tend to possess even nutraceutial benefits that can be consumed as food and medicine. The study is the first report on the *in vitro* antimicrobial analysis of the underutilized *E. tectorius* fruits. However, further studies are required in order to isolate the compounds responsible for its active potential, characterization and structural elucidation to find the mechanism of action and screen the possible synergistic activity among various compounds.

Conflicts of interest

None declared.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101260.

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