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Original article In-vitro antibacterial, antioxidant potentials and cytotoxic activity of the leaves of Tridax procumbens



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1. Introduction

ABSTRACT

The present study explored the phytochemicals, antibacterial, antioxidant and cytotoxic effect of *Tridax procumbens* leaves. The leaves were dried and extracted with various organic solvents. The leaves contained the phytochemicals such as alkaloids, carbohydrates, polyphenols and tannins respectively. Antimicrobial potentials of the extracts were determined by performing the disc diffusion techniques. Results revealed that different organic solvents extracts namely methanol, ethanol and ethyl acetate extracts documented comparatively good activity against the studied microbial strains. The methanol extract of leaves of *T. procumbens* showed combatively better antioxidant potential. The tested plant leaf extract showed high activity against human lung cancer cells than breast cancer cell lines. 250 μ g/ml plants extract showed 84 ± 2.8% toxicity against human lung cancer cells.

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Pathogenic bacteria always developing resistance against various antibacterial drugs used presently to control many diseases (Al-Dhabi et al., 2015; Al-Dhabi and Arasu, 2016; Barathikannan et al., 2016). For this reason, biologist, chemist and pharmacologist always were trying to explore novel compounds from various natural sources. The increasing availability of medicinal plants throughout the world attracted much more attention, because these medicinal plants have various useful metabolites (Bonjar and Farrokhi, 2004; Cuong et al., 2017; Elango et al., 2017). The parts of medicinal plants such as, root, stem, flowers, leaves possess various antimicrobial properties (Elango et al., 2016; Fowsiya et al., 2016 Glorybai et al., 2015; Haritha et al., 2016). It is widely used in traditional medicine system, as insect repellent, anticoagulant, antifungal and antibacterial agent (Helan et al., 2016; Ilavenil et al., 2017; Park et al., 2016a). Also, this medicinal

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plant promotes growth of hairs and showed wound healing property (Park et al., 2016b). The leaf extract showed antiparasitic activity and insecticidal properties (Ravikumar et al., 2005; Park etal., 2017). This medicinal plant showed the presence of phytochemical compounds such as, β-amyron, β-amyrin, stigma sterol, lupeol, luteolin, campasterol, arachidic acid, fucosterol, palmatic acid and lauric acid (Surendra et al., 2016a; Surendra et al., 2016b; Gurusamy et al., 2019). The knowledge of traditional medicine system and combined efforts between traditional healers and modern researchers are very important to validate existing knowledge of medicinal plants in a particular geographical location (Hamill et al., 2000). In under developing and developing countries, people frequently used traditional medicine system, however, little information is available on biological role and chemical composition of medicinal plants (Tabuti et al., 2003). Herbal medicines have been used in various countries to meet health care needs (Awe and Omojalasola, 2003; Surendra et al., 2016c; Roopan et al., 2019). Medicinal plants showed various activities, including, antimicrobial activities (Abo et al., 1999). Tridax procumbens has immense antibacterial and antifungal potential (Ali et al., 2001; Rajkumari et al., 2019; Valsalam et al., 2019a; Valsalam et al., 2019b) This medicinal plant is widely distributed in Asia, Africa and Australia and available in almost all seasons in almost all part of the country. Traditinally, Tridax procumbens has been used to treat typhoid fever, fever, cough, epilepsy, asthma, and diarrhoea

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(Nino et al., 2006). For the past few decades, the application of traditional system of medicine has increasing rapidly and the popularity also increasing. Traditional medicine system serves more than 80% of health needs. Among the therapeutic properties of medicinal plants, search of novel antimicrobial compounds is an urgent need because of emergence of drug resistance of bacteria (Peterson and Dalhoff, 2004). Although many medicinal plants have been evaluated for antibacterial potential for various biological activities, however these were not validated (Balandrin et al., 1985).

2. Materials and methods

2.1. Collection of medicinal plants and extraction of antimicrobial compounds

The leaves of *Tridax procumbens* were collected from Western Ghats, Tamilnadu, India. The collected *T. procumbens* were transported to the laboratory for identification and a specimen was stored for further studies. It was shade dried for 10 days and powdered using a mixer grinder. The leaf powder was used for the extraction of phytochemicals.

2.2. Phytochemical analysis

Leaves were subjected for the analysis of phytochemicals from medicinal plant by standard method. The solvent such as, methanol, ethanol, chloroform and water were used for the extraction of phytochemicals (Obadoni and Ochuko, 2001). About 75 g of dried powder was extracted with 300 ml of solvent in the increasing order based on their polarity for 48 h using a soxhlet apparatus as described previously (Bobbarala et al., 2009). The extract was further dissolved in dimethyl sulphoxide (10%) and stored in a vial for further studies.

2.3. Antimicrobial activity of T. Procumbens

Antibacterial activity of leaves of *T. procumbens* was evaluated by well diffusion method. The bacteria such as, *Escherichia coli*, *Bacillus subtilis*, *Staphylococus aureus* and *Enterobacter aerogenes* were used. Inoculum was prepared using nutrient broth medium (Himedia, Mumbai, India) and 10⁵ cfu ml⁻¹ bacterial cultures was spread on Mueller Hinton Agar plates and 6 mm diameter well was punched into the agar and filled with 40 µl (25 mg/ml) of crude extract. The inhibition zone around the sample well was measured (mm) (NCCLS, 1993).

2.4. Antioxidant properties of T. Procumbens

2.4.1. Hydrogen-donating activity

In the present study, DPPH (0.1 M) was used along with 50 μ l of methanol extract which was previously diluted with methanol at various concentrations (25–125 μ g/ml). Reaction was performed at room temperature for 30 min and the absorbance of the sample was read at 517 nm against the reagent blank. Percentage antioxidant activity (%AA) was calculated using ascorbic acid as the standard. The changes in colour indicate the scavenging activity of the extract.

2.4.2. Reducing power assay

For this assay plant extract and ascorbic acid (Sigma, USA) was dissolved in double distilled water and sodium phosphate buffer (0.2 M, pH 6.6). To this potassium ferricyanide (1%, v/w) was added and the mixture was incubated for 20 min at 50 °C.

2.5. Phytochemical profiling of the extract using Gas Chromatography-Mass Spectrometry analysis

The methanol extract of leaves sample was used for the determination of compounds using Gas Chromatography-Mass Spectrometry (GC–MS) analysis. Column Elite-1 fused silica capillary column. The injected sample was run for 30 min and the detected compounds were identified using NIST library.

2.6. Cytotoxic effect of methanol extract against human lung cancer cells-A549 and breast cancer cell lines-MCF-7

In the present study, the cytotoxic potential of methanol extract of leaf of *T. procumbens* was tested against human lung cancer cells- A549 and breast cancer cell lines. In Methyl tetrazolium (MTT) assay method 3×10^4 cells/well were inoculated with tissue culture medium in 96-well micro titre plate. The microtitre plates were incubated for overnight. After that, methanol extract was diluted at five different concentrations (50, 100, 150, 200 and 250 µg/ml) and treated with cell lines for 24 h. Then 20 µl MTT (5 mg/ml) was added to each microtitre plate (pH 4.7) and incubated for 4 h. After that the supernatant was carefully removed and DMSO was added in all wells and was well shaken for 20 min. The microtitre plate without cells is considered as blank. The absorbance of the sample was read at 570 nm against blank.

3. Results and discussion

3.1. Phytochemicals of the leaves of T. Procumbens

In the present study, active principles were extracted from T. procumbens and screened for various phytochemicals. The selected leaves showed the presence of phytochemicals such as, glycosides, flavonoids, saponins, steroids, alkaloids, carbohydrates, polyphenols and tannins. The methanol extract showed the presence of flavonoids, alkaloids, carbohydrates, polyphenols and tannins. The ethanol extract showed the presence of glycosides, flavonoids, carbohydrates, polyphenols and tannins. Water extract showed the presence of glycosides, flavonids, saponins and polyphenols (Table 1). Earlier, Tridax procumbens has been declared as the medicinal plant to treat various diseases (Taddei et al., 2000). Also, various bioactive compounds have been isolated and characterized from T. procumbens. The phytochemicals such as, alkaloid, tannin, coumarin and saponin have been detected from this plant (Prasad et al., 2008). In a study, Sawant and Godghate (2013) detected phytochemicals such as, saponin, steroid, diterpenes, alkaloids, flavonoids, phenol and tannins in the leaf of Tridax procumbens.

3.2. Antibacterial activity of leaves of T. Procumbens

The antibacterial activity of medicinal plants has been reported by various researchers, however, the research has been intensified for the past three decades. During these times, various antimicrobial properties have been reported from various traditional medicine system such as, Asian, Chinese and African (Palombo and Semple 2001). In the present investigation, the methanol and ethyl acetate extract showed good activity against selected pathogenic bacteria (Fig. 1). In plants, the secondary metabolites such as, flavonoids are critically active against many pathogenic organisms (Lutterodt et al. 1999). Also, plant based antibacterial agents have various therapeutic properties with little or no side effects (Lee et al., 1999). Also, studies revealed that *Tridax procumbens* leaves pentacyclic triterpenes (Gadre and Gabhe, 1992). Many researchers also have been reported similar results from various medicinal

Table 1Physiochemical analysis of leaves extract from Tridax procumbens.

Tests	Methanol	Ethanol	Chloroform	Aqueous	Acetone	Ethyl acetate
	Extract	Extract	Extract	Extract	Extract	Extract
Glycosides	-	+	-	+	-	+
Flavonoids	+	+	+	+	+	-
Saponins	-	-	_	+	-	-
Steroids	_	-	_	-	-	-
Alkaloids	+	-	+	-	+	+
Carbohydrates	+	+	_	-	-	-
Polyphenols	+	+	_	+	-	-
Tannins	+	+	+	-	-	-



Fig. 1. Antibacterial activity of leaves extract against pathogenic bacteria. 1chloroform extract, 2- water extract, 3-ethyl acetate extract, 4- methanol extract, 5acetone extract and 6 – ethanol extract.

plants (Thomas and Mccubbin 2003; Sasikumar et al., 2007). Traditionally, the stem of Tridax has been very much associated with antimicrobial activity (Mundada and Shivhare, 2010). Previous finding revealed antibacterial activity of T. procumbens extracted with ethanol, however water extract did not show any antibacterial activity (Aniel and Naidu, 2010). The variation in antibacterial activity between alcohol extract and aqueous extract showed the fact that various solvents have different capacities to extract phytochemicals based on their polarity and solubility. The antimicrobial properties of Tridax mainly due to the presence of flavonoids, alkaloids, saponins and tannins (Mundada and Shivhare, 2010). Medicinal plants provide various lead molecules to cure and to treat various disorders and diseases. These lead molecules can be used to develop various phytomedicine from the traditional medicine system such as, Ayurvedic and Unani. These traditional medicine system forms blueprint for the development of new drugs (Didry et al., 1998).

3.3. Antioxidant activity of T. Procumbens

3.3.1. Reducing power of plant extract

In this sample the reaction was found to be high indicating high reducing power. In this study, the absorbance of ascorbic acid was treated as 100% antioxidant potential. Table 2 shows the antioxidant potential of methanol, aqueous, ethanol and chloroform

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Antioxidant activity of leave	s extract of Tridax procumbens.
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Particulars	Absorbance of the sample (700 nm)	Antioxidant activity (%)
Methanol Ethanol Aqueous Chloroform Acetone Ethyl acetate Standard	$\begin{array}{c} 0.457 \pm 0.023 \\ 0.539 \pm 0.018 \\ 0.263 \pm 0.28 \\ 0.224 \pm 0.32 \\ 0.412 \pm 0.22 \\ 0.252 \pm 0.101 \\ 0.876 \pm 0.421 \end{array}$	52.16 ± 0.21 61.52 ± 0.32 30.02 ± 0.41 25.57 ± 0.38 47.03 ± 2.7 28.75 ± 3.2 100
Standard		100

extract of leaves of T. procumbens. Among all extracts, ethanol extract showed considerable antioxidant activity ($61.52 \pm 0.32\%$), whereas, aqueous extract showed least antioxidant activity ($25.57 \pm 0.38\%$). Previously various research groups reported flavonoids and phenolic contents of some medicinal plants and antioxidant activity.

3.3.2. Hydrogen-donating activity of leaves extract from Tridax procumbens

Table 3 shows the DPPH radical scavenging activity of leaves of ethanol extract from T. procumbens at various concentrations. The DPPH results were comparatively less than that of standard ascorbic acid. The ethanol extract used in this study showed DPPH radical scavenging potential. DPPH scavenging activity was $6.2 \pm 0.2\%$ at 25 µg/ml and showed 82.5 ± 1.1% activity at 200 µg/ml (Table 3). The present results support previous findings that secondary metabolites such as, tannins, phenolic compounds, catechins and flavonoids are responsible for antioxidant activity. These antioxidant compounds showed preventive activity against heart disease, age related diseases, development of cancer, repairing tissue damage and lowering blood cholesterol. Recently Andriana et al. (2019) reported antioxidant properties of ethanolic extract of *Tridax procumbens* L.

3.4. Gas Chromatography-Mass Spectrometry analysis

Methanol extracts showed 9 peaks and the compounds were identified using NIST database. The identified compounds were,

Table 3			
Antioxidant activity of T.	procumbens	leaves	extract.

Ethanol extract con (µg/ml)	DPPH Scavenging activity (%)
25	6.2 ± 0.2
50	10.40 ± 4.3
75	11.90 ± 2.8
100	25.2 ± 5.2
125	53.8 ± 2.01
150	75.2 ± 0.38
175	79.1 ± 1.2
200	82.5 ± 1.1

n-Hexadecanoic acid, Tetradecanoic acid, Tridecanoic acid, Octadecanoic acid, Caryophyllene, *trans*- α -Bergamotene. The detected compounds have various therapeutic potentials. These compounds have anticancer, immune suppressive, anti-spermatogenic, antineoplastic, antiarthritic, anti-inflammatory and antimicrobial activity (Ramesh et al., 2013).

3.5. Cytotoxic effect of methanol extract against human lung cancer cells-A549 and breast cancer cell lines-MCF-7

Cytotoxicity of methanol extract of T. procumbens against human lung cancer cells and breast cancer cell lines were assayed. MTT assay was performed to analyze cytotoxic effect. The tested plant leaf extract showed high activity against human lung cancer cells than breast cancer cell lines. 250 µg/ml plants extract showed 84 ± 2.8% toxicity against human lung cancer cells (Fig. 2), however. only 68 ± 3.1% cytotoxicity was obtained in breast cancer cell lines (Fig. 3). Cytotoxic effect increased at increasing concentration of plant extract. Previously the cytotoxic potential of acetone and aqueous extract from the flower and leaf extract of T. procumbens has been described against prostate epithelial cancer (PC3) cell line (Priva et al., 2011a and b). In their studies they used acetone extract and 250 µg/ml plant extract showed 82.28% cell death which was less than our findings ($84 \pm 2.8\%$ cell death). However, the phytochemical property of the medicinal plant may vary based on geographical locations and extraction methods (Rai et al., 2013). This medicinal plant has phytochemicals such as, oleanolic acid and its derivatives were highly effective against MCF-cancer cell



Fig. 2. Cytotoxic effect of *T. procumbens* methanol extract against human lung cancer cells- A549. Cytotoxic effect was tested at five different concentrations ranging from 50 to 250 μ g/ml.



Fig. 3. Cytotoxic effect of *T. procumbens* methanol extract against breast cancer cell lines-MCF-7. Cytotoxic effect was tested at five different concentrations ranging from $50 - 250 \mu$ g/ml.

lines. The phytochemicals from this plant arrested cell cycle and has been reported previously (Allouche et al., 2010; Lucio et al., 2011). Likewise, oleanolic acid and its derivatives showed cytotocic effect against breast cancer cell lines (Akl et al., 2014). In another study, the bioactive compound, betulin is non-toxic to cells; however betulinic acid was reported for its anticancer potential (Kim et al., 1997). Luteolin has free-radical scavenging ability, because of this effect, anti-cancer property was detected previously (Seelinger et al., 2008). It also suppressed angiogenesis and showed activity against various types of cancer cell lines, including, uterine cancer, colon cancer, liver cancer and breast cancer (Makino et al., 1998; Leung et al., 2006; Pettit et al., 1996; Tu et al., 2013).

In conclusion, the tested plant leaf extract showed high activity against human lung cancer cells than breast cancer cell lines. 250 μ g/ml plants extract showed 84 ± 2.8% cytotoxicity against human lung cancer cells and 68 ± 3.1% cytotoxicity was obtained in breast cancer cell lines. The detailed study of the phytochemicals and structural elucidation of the compounds will contribute to the discovery of novel drugs.

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