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Antibacterial potential and phytochemical analysis of two ethnomedicinally important plants

Narpat Singh, Neha Chhangani, Sharad Bissa *

Microbiology and Plant Pathology Laboratory, Department of Botany, Jai Narain Vyas University, Jodhpur, Rajasthan, India

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ABSTRACT

Medicinal plants exhibited great role in drug industries. Herbal medicines and their derivative products are often prepared from crude plant extracts. *Echinops echinatus* and *Tridax procumbens* both are belonging to Asteraceae family and these plants are ethnomedicinally important due to their utilization as traditional medicine to cure various diseases. Aim of the current study is to evaluate the antimicrobial properties, preliminary phytochemical and GC-MS analysis of these ethnomedicinally important plants to identify the compounds which are responsible for antimicrobial properties. Their extracts exhibited antimicrobial activity against *Enterobacter aerogenes, Escherichia coli, Agrobacterium tumefaciens, Staphylococcus aureus, Bacillus subtilis, Pseudomonas syringae* and *Pseudomonas putida*. Both plants contain the active principles like flavonoids, alkaloids, glycosides, saponins, terpenoids and tannins. Result of GC-MS analysis showed the presence of many compounds such as n-Hexadecanoic acid, Hexadecanoic Acid, Methyl ester, Octadecanoic acid, Stigmasterol, Naphthalene, Squalene, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, Squalene, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-,5 Hydroxymethylfurfural, Lupeol, Dodecanoic acid, Vitamin E (α-Tocopherol), Neophytadiene, Phytol and many other compounds. These compounds are responsible for antimicrobial, anticancer and medicinal properties.

Introduction

Plants are important source for the treatment of various diseases. The bioactive compounds which are obtained from plants are known as phytochemicals. *Echinops echinatus* (popularly known as "Uthkanta"), belongs to the Asteraceae family and distributed worldwide except Antarctica (Funk et al.,2005; Benvidi and Jahanbin,2020). This genus includes about 120–130 species and found in the Mediterranean, Africa and central Asia (Sánchez-Jiménez et al.,2010). It is an erect, rigid, pubescent, annual herb about 1 meter in height. It has short, stout stems with branches spreading widely from the base. The leaves are alternately arranged, sessile, oblong, deeply pinnatified, 7-12 cm long, and covered with cottony wool beneath; the lobes are triangular, simulate and prickly, and the spines are often 2.5 cm long. Flower heads occur in solitary white spherical balls, 3-5 cm across, clustered at the ends of branches. Flowers are surrounded by strong white bristles resembling pappus hairs; the pappus is short, yellowish, and forms a short cylindrical brush above the achene. Petals of the tiny white flowers are 5 mm long. Flowering occurs between December and January. Seeds are sweet and aphrodisiac.

Tridax procumbens, is a perennial plant, commonly known as "coat buttons" belongs to the Asteraceae family, native to Central and South America (Hilliard, 1977; Ravikumar et al., 2005). It is present in semi prostrate habit and can grow anywhere up to 15-40 cm in height. Roots of *T. procumbens* arising at the nodes; stems procumbent, hairy and arising from woody base; leaves are elongated, opposite, ovate with serrated margins, abaxial and adaxial sides covered with fine whitish hairs; ray florets and disk florets are two types of flowers with basal placentation 3–6, tubular at the base with pale yellow or cream-white ligules, 2.5–5 mm long, 2–5 mm wide with the yellow disk of corollas (Powell, 1965). Fruit is a hard achene covered with stiff hairs and has a plume, feathery like white pappus at the one end. The calyx is represented by scales or reduced to pappus. Seed has pendulous endosperm and the embryo is absent. (Rahman et al., 2008; Chauhan, B.S. and Johnson, D.E., 2008).

* Corresponding author
E-mail address: bissasharad@yahoo.co.in (S. Bissa).

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Table 1
Fresh extracts of *Echinops echinatus*.

Plant Part	Plant Extract	Zone of Inhibition (mm)						
		E. coli	*E. aerogenes*	*A. tumefaciens*	*B. subtilis*	*S. aureus*	*P. putida*	*P. syringae*
Leaves	Aqueous	-	1.67±0.58	-	-	1.33±1.15	1.67±1.53	2.67±2.31
	Alcoholic	4.33±0.58	7±1	4±1.73	5.33±0.58	2.33±1.15	4.67±0.58	2±1
	Chloroform	1.33±0.58	2.33±1.15	5.33±0.58	4±2	4.67±2.08	3.67±1.15	5±1
	Petroleum ether	3.67±1.15	7.33±2.08	7.67±1.15	2.33±1.15	2.67±0.58	4.33±1.15	7±1.73
Flowers	Aqueous	-	2.33±0.58	-	2.67±0.58	-	-	2±1
	Alcoholic	4.67±1.15	2.67±0.58	1.67±0.58	4±1	3.33±0.58	-	3.33±1.53
	Chloroform	6±1.73	4.33±1.15	4±1	6.67±1.53	5.33±1.53	2.33±1.15	5±2
	Petroleum ether	7±1	8.67±1.15	6±1.73	6±1	4±1	3.67±2.31	4.33±0.58
Stem	Aqueous	1.67±0.58	5.67±2.08	2.33±0.58	2±1.73	-	1.67±0.58	2.33±0.58
	Alcoholic	2.67±2.08	3.67±1.53	3.33±1.53	4.33±1.15	6±1	3.33±0.58	5±1.73
	Chloroform	6.67±2.31	5±1	3±1.73	7.67±0.58	4.67±1.15	5±1	5.33±1.15
	Petroleum ether	7.33±1.15	7±1.73	6.33±0.58	6.67±1.15	4.67±0.58	4.33±0.58	7±1.73
Root	Aqueous	2.33±1.15	3.33±0.58	2.67±0.58	1.67±0.58	2.67±1.15	3.67±2.31	3±2
	Alcoholic	4±1	3.67±0.58	4.67±1.15	5±1	5.33±1.15	5.33±1.15	4.33±2.89
	Chloroform	5.67±0.58	5±1.73	4.33±0.58	4.67±1.15	6.33±0.58	7±1	5.33±1.53
	Petroleum ether	5±1.73	8±2	6.33±1.15	5.33±1.53	7.33±1.15	9.33±0.58	8.67±1.15

Table 2
Dry extracts of *Echinops echinatus*.

Plant Part	Plant Extract	Zone of Inhibition (mm)						
		E. coli	*E. aerogenes*	*A. tumefaciens*	*B. subtilis*	*S. aureus*	*P. putida*	*P. syringae*
Leaves	Aqueous	-	-	1.67±0.58	-	-	3.0±1.73	3.33±1.53
	Alcoholic	3.67±2.31	3.67±1.53	6.33±1.53	4±1.73	3.67±1.15	4.33±1.15	4±1.73
	Chloroform	7.67±1.53	6±1	9.33±2.89	6±1	5.33±0.58	5±1	7.33±2.08
	Petroleum ether	10±1	9.33±1.53	10.67±2.08	9.33±0.58	8.33±1.55	7.67±1.15	9.67±1.15
Flowers	Aqueous	1.67±0.58	3.33±1.15	3±1	3.67±1.53	3±1	3.33±0.58	2±1
	Alcoholic	5.33±1.53	4.67±0.58	2.67±1.15	5.33±1.53	5±1	8±1.73	5.33±0.58
	Chloroform	9.33±2.08	9.67±1.53	7.33±0.58	10.33±1.15	6.67±0.58	10.67±0.58	3.33±1.15
	Petroleum ether	11.33±1.53	10.33±0.58	5.33±1.15	12±1	9.67±1.15	11±2	6±1
Stem	Aqueous	5.33±0.58	3.67±1.15	4±1.73	-	-	4.33±1.15	3.33±0.58
	Alcoholic	7.67±1.15	4.67±1.15	7.33±1.15	3.67±1.15	1.67±0.58	7.67±2.31	5.67±1.15
	Chloroform	8±1.73	7.33±1.53	7±1.73	4.33±0.58	5.33±2.31	6±1.73	6.33±1.15
	Petroleum ether	9.33±0.58	7±2.65	8.67±0.58	6.67±2.52	8.33±0.58	5.33±1.53	6±1
Root	Aqueous	4.67±1.15	1.67±0.58	2.33±0.58	3.67±1.15	4.67±0.58	3.33±2.52	3±1
	Alcoholic	6.33±0.58	8±1.73	5.33±1.15	5.67±0.58	7±1	4.33±0.58	3.67±0.58
	Chloroform	5±1.73	9±1	7.33±0.58	6.67±0.58	9.67±1.15	7.67±2.31	10.33±1.15
	Petroleum ether	8.33±1.15	12.67±1.53	9±2	6±2.65	9.33±0.58	9.67±0.58	13±2

Ethnomedicinal Uses

According to Nadkarni (1976), the root powder of *E. echinatus* is very useful in treating coughs and fever in children. Many Researchers including Rathore et al (2015); Ghasemi et al (2013); Regassa (2013) observed that this genus has therapeutic benefits including the improvement of some illness symptoms like pain, inflammation, fever, respiratory tract illnesses, sore throat and cough etc. Issar (1974) reported that the roots of this plant are commonly available in markets under the trade name of brahmadandi. Traditionally *E. echinatus* is used against many diseases like stomach disorder, antipyretic, eczema and it also possesses antibacterial, antioxidant, anthelmintic and antidiuretic properties (Arshad et al., 2002; Parrotta, 2001). According to Aslam et al. (2015), crushed root of *E. echinatus* with gum of *Acacia* is applied on hair to destroy lice.

E. echinatus is the useful traditional medicinal plant in India. Various parts of the *E. echinatus* have been investigated due to presence of several phytochemicals (Aslam et al.,2015). Benvidi and Jahanbin (2020), observed the chemical composition of some species of *Echinops* indicates the presence of benzothiopheneglycoside, flavone, alkaloids, polyacetylene thiophenes, and carbohydrates. Singh et al (1989), reported that the extract of *E. echinatus* inhibits the acute inflammation induced by carrageenan, formaldehyde and the chronic arthritis induced by formaldehyde in rats and studies also suggests that the oral extract was more effective.

T. procumbens has diverse pharmacological properties including immunomodulatory, anti-oxidant, antihepatotoxic, analgesic, antidiabetic, anti-inflammatory, antifungal, and antimicrobial activities (Ravikumar et al., 2005a; Ravikumar et al., 2005b; Bhagwat et al., 2008; Sawant et al., 2014; Hitesh, 2006). It is also used in the treatment of anaemia, jaundice, colds, inflammation, and hepatopathies in Central America (Taddei and Rosas Romero, 2000; Saraf and Dixit, 1991). The whole plant is used for the treatment of fever, cough, typhoid fever, stomachache, backache, diarrhea, epilepsy and protozoal infections (Soladoye et al., 2013; Mann et al., 2003. Decoction of the leaves is used against pain, to treat malaria, diabetes and gastrointestinal mycosis (Agban et al., 2013; Pareek et al., 2009; Pardeshi and Bhiungade, 2016). *T. procumbens* is known as an insect repellent, used to treat diarrhoea, scrutinize hemorrhages, cure for hair loss, wounds and stops bleeding. (Policegoudra et al.,2014; Saraf et al.,1990; Caceres et al.,1998).

Antimicrobial Activity

E. echinatus has prominent phytochemicals and showed antibacterial activities against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia* and also possess anti-urease inhibition assay activities (Rafay et al 2021).

Crude extracts of *T. procumbens* showed antimicrobial activity against *P. aeruginosa, B. subtilis, E. fecalis* and *E. coli*. Flower extract of n-hexane showed antimicrobial activity against *Mycobacterium smegmatis, Escherichia coli, Klebsiella sp., Salmonella group C, Salmonella paratyphi* while ethyl acetate extract showed the zone of inhibition against *Bacillus*

cereus and *Klebsiella sp*. (Taddei and Rosas-Romero 2000). The ethanolic extract have shown significant activity against *Staphylococcus aureus* (Ayyappa Das et al. 2009). Essential oil extract showed significant activity against Gram positive bacteria: *Staphylococcus aureus* and *Streptococcus pneumoniae* (Manjamalai et al., 2012b).

A-B *Echinops echinatus* in their Natural habitat and flowering stage C-D- *Tridax procumbens* in their Natural habitat and flowering stage

Materials and Methods

Collection of plant material- Fresh and green different plant part of *E. echinatus* and *T. procumbens* were collected from Aravali range of Pali district in Rajasthan. Their identity was confirmed from the literature available in Department of Botany, J.N.V University, Jodhpur. Different plant parts viz. leaves, flower, pods, stem, seed and bark were thoroughly washed under running tap water followed by distilled water to remove attached litter and soil debris and then dried under shade at 28± 2◦C for about 10 days. The dried plant samples were ground well into a fine powder in a mixer grinder and sieved to give particle size of 50–150mm. The powder was stored in air sealed polythene bags at room temperature before extraction. 25g of dried powder was packed in a Whatmann filter paper no.1 and was extracted in a soxhlet apparatus using 100ml of solvent. Solvents used for the extraction were petroleum ether, chloroform, ethanol and aqueous and the extracts were dried. The dried extracts were stored in a refrigerator at 40◦C. Finally, the concentration of 5 mg per disc was loaded on each disc.

Antibacterial Susceptibility Test- All the plant part extracts were screened against *E. coli, Enterobacter aerogenes, Agrobacterium tumefaciens, Bacillus subtilis, Pseudomonas putida* and *Pseudomonas aeruginosa* pathogenic bacterial strain. The disc diffusion method was used to test

Table 3
Fresh extracts of *Tridex procumbens*.

Plant Part	Plant Extract	Zone of Inhibition (mm)						
		E. coli	*E. aerogenes*	*A. tumefaciens*	*B. subtilis*	*S. aureus*	*P. putida*	*P. syringae*
Leaves	Aqueous	2±1	-	2.67±0.58	1.67±0.58	-	-	3.33±1.53
	Alcoholic	4.33±1.15	3±1	4±1.73	3.33±1.53	2.33±1.15	2.33±0.58	4±1.73
	Chloroform	5.33±1.53	4.67±2.08	4.67±1.15	5.33±0.58	3.67±1.15	6.33±1.15	7.33±0.58
	Petroleum ether	6±1.73	4.33±1.15	5.67±2.52	8.67±0.58	5.33±1.53	4.67±1.53	8±1
Flowers	Aqueous	2.33±1.53	2.33±0.58	2±1.73	3.33±1.53	2.67±0.58	3±1.73	2.67±2.31
	Alcoholic	4.33±0.58	3.33±1.15	4.67±0.58	3.67±1.15	4.33±1.15	2.33±1.15	3.67±2.31
	Chloroform	6.67±1.15	4.33±1.15	6±1	6.67±1.53	5±1	4.33±0.58	7±2
	Petroleum ether	6.33±1.53	6±1.73	9±4	6±1.73	6.33±1.15	7.67±0.58	6.67±1.53
Root	Aqueous	1.33±0.58	-	-	1.67±0.58	1.67±1.53	2.67±0.58	1.67±0.58
	Alcoholic	3.67±2.31	2.67±1.15	-	2±1	5.33±2.08	4.33±1.15	4±1.73
	Chloroform	4.67±1.53	4.67±4.16	3.67±1.15	7.67±0.58	6±1	8±1.73	5.33±0.58
	Petroleum ether	4.33±2.31	5±2	4.33±0.58	5±1.73	6±1.73	7.67±2.08	6.33±1.15

Table 4
Dry extracts of *Tridex procumbens*.

Plant Part	Plant Extract	Zone of Inhibition (mm)						
		E. coli	*E. aerogenes*	*A. tumefaciens*	*B. subtilis*	*S. aureus*	*P. putida*	*P. syringae*
Leaves	Aqueous	3.67±1.15	2.67±1.53	3±1.73	2.33±1.15	2.67±0.58	3.67±0.58	3.33±1.15
	Alcoholic	5.67±2.31	5.33±1.53	5.67±2.31	3±1.73	4.67±1.53	4.33±1.15	8.67±1.15
	Chloroform	8.33±1.15	9.33±2.08	9±2	3.33±0.58	7.67±2.31	10.67±2.52	9±1.73
	Petroleum ether	10±1	12.33±1.15	9.67±1.15	4±1	8.67±2.08	6±1.73	10.67±2.52
Flowers	Aqueous	3±1.73	2±1	2.33±0.58	-	3.67±1.15	2.33±1.53	4±2.65
	Alcoholic	5.67±0.58	5.67±1.53	-	4.33±0.58	5.33±1.53	3.33±1.15	8±1.73
	Chloroform	7.33±1.15	4±1	3.67±0.58	6.33±2.08	6.67±1.53	4±1	9.67±1.15
	Petroleum ether	6.67±2.08	8.33±2.52	5±1	5.67±1.53	8±3	5±2	9.33±1.53
Root	Aqueous	3.67±1.15	2±1	3±1.73	3±2	3.67±1.53	3±1.73	3.33±1.15
	Alcoholic	5.67±1.15	6.33±1.15	6.67±0.58	6.67±0.58	6.33±1.15	6.33±1.53	4.33±1.15
	Chloroform	6±1.73	5.33±2.52	9±2	7.67±1.15	8±1	8.33±0.58	7.33±2.08
	Petroleum ether	7.67±1.15	5±1	6.33±1.15	10.33±1.15	11±2	10.67±2.52	7.67±0.58

the antimicrobial activity of the plant extracts (Bauer et al 1966). 20ml of sterilized nutrient agar medium for pathogens were poured into each sterile petri dish. The plates were allowed to solidify for 5 minutes and 0.1% inoculum suspension was swabbed uniformly. The entire agar surface of each plate was inoculated with swab, first in the horizontal direction and then in a vertical direction, which ensures the even distribution of organism over the agar surface. The filter paper discs (5mm in diameter) loaded with 5 mg/ disc of dry extract were placed on the surface of the bacteria seeded agar plates and the compound was allowed to diffuse for 5 minutes and then the plates were incubated at 37°C for 24h. After 24 hours, the inhibition zones formed around the disc were measured with transparent ruler in millimeter. These studies were performed in triplicate.

Phytochemical analysis

I. **Collection of plant material**

Fresh plant materials were collected from the field. Plants were then brought to the laboratory and thoroughly washed under running tap water followed by distilled water to remove attached litter and soil debris. Then shade dried for 10-15 days. These dried plant materials were then stored in an airtight container till further use.

II. **Preparation of Plant extracts**

10 gm of dried plant powder dissolved in 100 ml of distilled water and methanol for 48-72hrs. The samples were filtered using muslin cloth. The solvent distilled water and methanol were evaporated to semisolid form by using water bath (Mahida and Mohan 2007).

Table 5
Quantitative Phytochemical analysis of *E. echinatus*..

Name of Plant Part	Alkaloids	Glycosides	Flavonoids	Saponins	Tannins	Terpenoids
Leaves	-	+	-	+	+	+
Flower	+	+	+	+	-	+
Root	+	+	+	+	-	-
Stem	-	+	-	+	-	+

Table 6
Quantitative Phytochemical analysis of *T. procumbens*.

Name of Plant Part	Alkaloids	Glycosides	Flavonoids	Saponins	Tannins	Terpenoids
Leaves	+	-	+	-	+	+
Flower	+	-	+	+	+	+
Root	-	+	+	-	-	-

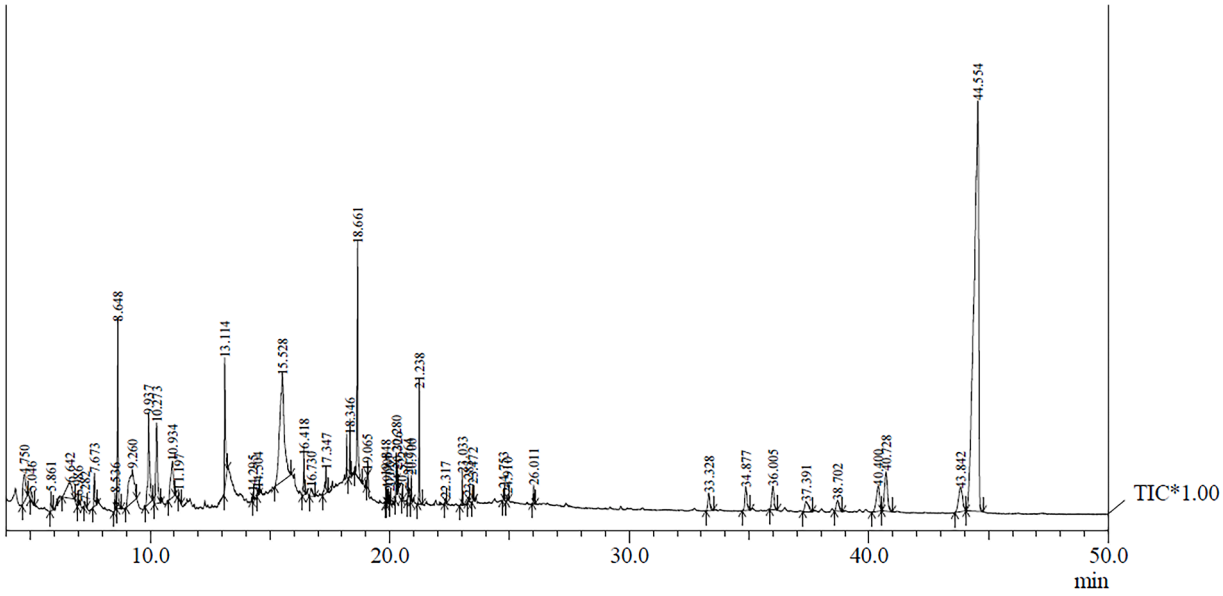


Fig. 1. Chromatogram of methanolic root extract *Echinops echinatus*.

Preliminary Phytochemical screening

The supernatant obtained from various solvents were used as the plant extract and were subjected to qualitative phytochemical screening for the identification of different classes of chemical constituents following standard methods (Harborne and Harborne, 1973).

i. **Test for alkaloids**
   Dragendroff's test: 2 ml of extract was taken in a test tube and acidified with 2 ml of 1% hydrochloric acid, then a few drops of freshly prepared Dragendroff reagent was added slowly to the solution. The appearance of an orange-brown precipitate indicated the presence of alkaloids.
ii. **Test for glycosides**
   Keller-Kilani test: 3 ml of plant extract was treated with 2 ml of glacial acetic acid followed by the addition of 1-2 drops of 2% ferric chloride solution. The mixture was then poured into the test tube containing 2 ml of concentrated sulphuric acid. The appearance of a reddish-brown ring at the junction confirmed the presence of glycosides.
iii. **Test for flavonoids**
   Few drops of 2% aqueous sodium hydroxide solution were added to 2 ml of plant extract. The intense yellow colour appeared which becomes colourless on further addition of dilute hydrochloric acid indicated the presence of flavonoids.
iv. **Test for Saponins**
   2 ml of plant extract was diluted with 10 ml of distilled water in a test tube and shaken well for 15 minutes. The formation of a 1 cm. thick layer of foam indicated the presence of saponins.
v. **Test for Tannins**
   0.5g of the dried powered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green or a blue-black colouration.
vi. **Test for terpenoids**
   Salkowski test: 2 ml of plant extract was treated with 3 ml of chloroform solution and allowed to stand for few seconds, followed by the addition of 2 ml of concentrated sulfuric acid along the walls of the test tube. The formation of a reddish-brown ring at the interface of two liquids confirmed the presence of terpenoids.

GCMS analysis

Extract preparation for GC-MS analysis: 10 g of dried plant powder was extracted with 100 ml of HPLC grade methanol and kept in dark for 48 hours with occasional stirring. The extract was then filtered with Whatman filter paper No.1 centrifuged at 2500 rpm for 15 minutes. The obtained supernatant was evaporated on a water bath at 40°C to get the crude syrupy extract. For GCMS analysis crude extract was redissolved in methanol to make a stock solution. 1 µl of stock solution was used in GCMS analysis.

Identification of chemical compounds: The GCMS analysis of crude extract was performed with GCMS equipment QP 2010 Shimadzu, Japan. Experimental conditions for GCMS were as follows. Helium gas was used as the carrier gas at a constant flow rate of 16.3 ml. per min. and column flow rate 1.21 ml. per min. Injector and mass transfer line temperatures were 260 and 28$\mathring{0}$ C for 10 min. The total running time of GC-MS was 50 minutes. The injection volume was 1µl. As individual compounds eluted from the GC column where these compounds were bombarded with a stream of electrons causing them to break into a fragment, the samples were run fully at a range of 50/650 m/z and a mass spectrum graph was obtained which is a fingerprint of a molecule. The identified compounds were compared with the NIST library and Willey spectral library search program.

Observation and Results

Antibacterial activity

In genus *Echinops* the presence of secondary metabolites, such as phenolic compounds, tannins, and saponins have been considered to improve the anti-microbial efficacy of crude drugs against some disease-causing microorganisms to health promotion (Alizadeh Behbahani et al., 2020). *Piper betel* leaves have been shown inhibitory effect on oral microbiota (Bissa et al. 2007). Bissa and Bohra (2015), investigated the antibacterial efficacy against E. aerogenes. Religious plants have the potential to neutralize a variety of harmful pathogens with their antibacterial properties (Bissa 2018). Result for antibacterial activities in *E. echinatus* fresh leaves, flowers, stem and root extract in different solvent such as aqueous, alcoholic, chloroform and petroleum ether shown significant antibacterial activity against *Escherichia coli, Enterobacter aerogenes, Agrobacterium tumefaciens, Staphylococcus aureus, Bacillus subtilis Pseudomonas putida* and *Pseudomonas syringae*. In Table 1:

Table 7
list of compounds presents in methanolic root extracts of *E. echinatus*.

Peak#	R.Time	Area%	Molecular weight	Molecular Formula	Name
1.	4.750	1.65	180	$C_6H_{12}O_6$	dl-Glyceraldehyde dimer
2.	5.046	0.33	98	$C_5H_6O_2$	2(3H)-FURANONE, 5-METHYL-
3.	5.861	0.35	144	$C_6H_8O_4$	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
4.	6.642	1.89	92	$C_3H_8O_3$	Glycerin
5.	6.986	0.13	166	$C_5H_8O_3$	4-OXOPENTANOIC ACID
6.	7.282	0.13	128	$C_6H_8O_3$	2,5-ANHYDRO-1,6-DIDEOXYHEXO-3,4-DIULOSE
7.	7.673	0.80	126	$C_7H_{10}O_2$	Cyclopentane, 1-acetyl-1,2-epoxy-
8.	8.536	0.31	144	$C_6H_8O_4$	2-ACETYL-2-HYDROXY-.GAMMA.-BUTYROLACTON
9.	8.648	3.37	144	$C_6H_8O_4$	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
10.	9.260	3.71	92	$C_3H_8O_3$	1,2,3-PROPANETRIOL
11.	9.937	3.24	126	$C_6H_6O_3$	5-Hydroxymethylfurfural
12.	10.273	3.13	134	$C_5H_{10}O_4$	1,2,3-Propanetriol, 1-acetate
13.	10.934	2.16	144	$C_7H_{12}O_3$	Heptanoic acid, 6-oxo-
14.	11.197	0.18	144	$C_7H_{12}O_3$	4-PENTENOIC ACID, 3-HYDROXY-, ETHYL ESTER
15.	13.114	2.83	136	$C_8H_8O_2$	2.83 2,4-CRESOTALDEHYDE
16.	14.295	0.12	256	$C_{16}H_{32}O_2$	HEXADECANOIC ACID
17.	14.504	0.13	168	$C_{11}H_{20}O$	1-Cyclohexene-1-ethanol, 2,6,6-trimethyl-
18.	15.528	10.58	192	$C_7H_{12}O_6$	1,3,4,5-TETRAHYDROXY-CYCLOHEXANECARBOXY
19.	16.418	0.89	180	$C_{10}H_{12}O_3$	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol
20.	16.730	0.39	192	$C_7H_{12}O_6$	1,3,4,5-TETRAHYDROXY-CYCLOHEXANECARBOXY
21.	17.347	0.78	336	$C_{19}H_{32}N_2O_3$	1,5-Dimethyl-3,7-bis-(3-methylbutyryl)-3,7-diazabicyclo[3
22.	18.346	0.75	216	$C_{12}H_8S_2$	5-(But-3-ene-1-ynyl)-2,2′-bithienyl
23.	18.661	4.71	256	$C_{16}H_{32}O_2$	n-Hexadecanoic acid
24.	19.065	0.08	287	$C_{15}H_{29}NO_4$	l-Isoleucine, N-ethoxycarbonyl-, isohexyl ester
25.	19.848	0.33	294	$C_{19}H_{34}O$	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
26.	19.900	0.16	296	$C_{19}H_{36}O_2$	9-Octadecenoic acid (Z)-, methyl ester
27.	20.007	0.12	296	$C_{20}H_{40}O$	2-HEXADECEN-1-OL,3,7,11,15-TETRAMETHYL-
28.	20.280	0.42	280	$C_{18}H_{32}O_2$	9,12-Octadecadienoic acid (Z,Z)-
29.	20.326	0.16	238	$C_{16}H_{30}O$	cis-9-Hexadecenal
30.	20.522	0.13	284	$C_{18}H_{36}O_2$	Octadecanoic acid
31.	20.764	0.25	246	$C_{18}H_{30}$	Chrysene, octadecahydro-
32.	20.900	0.31	248	$C_{15}H_{20}O_3$	Santamarine
33.	21.238	1.73	248	$C_{12}H_8S_3$	[2,2′;5′,2"]TERTHIOPHENE
34.	22.317	0.09	256	$C_{13}H_{21}C_lOsi$	4-Chlorobenzyl alcohol, TBDMS derivative
35.	23.033	0.47	232	$C_{15}H_{20}O_2$	5-Isopropyl-2-methylphenyl 2-methylbut-2-enoate
36.	23.283	0.07	326	$C_{22}H_{46}O$	Behenic alcohol
37.	23.472	0.22	330	$C_{19}H_{38}O_4$	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl este
38.	24.753	0.18	272	$C_{15}H_{12}O_5$	7-(3,4-Methylenedioxy)-tetrahydrobenzofuranone
39.	24.910	0.10	266	$C_{18}H_{34}O$	9-Octadecenal, (Z)-
40.	26.011	0.30	410	$C_{30}H_{50}$	Squalene
41.	33.328	0.75	412	$C_{29}H_{48}O$	Stigmasterol
42.	34.877	1.09	414	$C_{29}H_{50}O$	STIGMAST-5-EN-3-OL, (3.BETA.,24S)-
43.	36.005	1.15	426	$C_{30}H_{50}O$	.beta.-Amyrin
44.	37.391	0.76	470	$C_{31}H_{50}O_3$	METHYL COMMATE B
45.	38.702	0.63	468	$C_{32}H_{52}O_2$	Olean-12-en-3-ol, acetate, (3.beta.)-
46.	40.400	1.78	442	$C_{30}H_{50}O_2$	Betulin
47.	40.728	2.53	426	$C_{30}H_{50}O$	Lupeol
48.	43.842	2.12	468	$C_{32}H_{52}O_2$	Lup-20(29)-en-3-ol, acetate, (3.beta.)-
49.	44.554	41.51	468	$C_{32}H_{52}O_2$	LUP-20(29)-EN-3-YL ACETATE

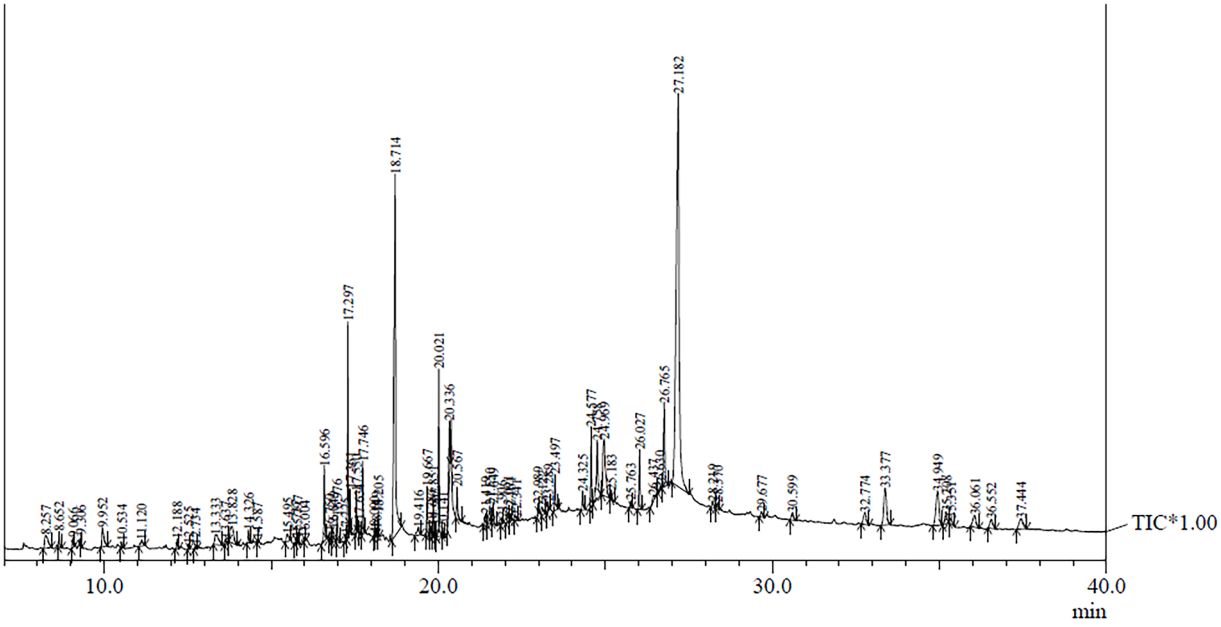
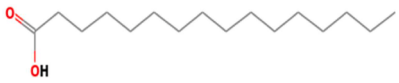
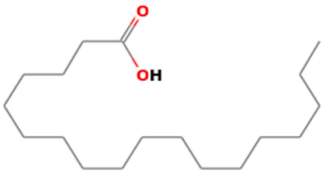
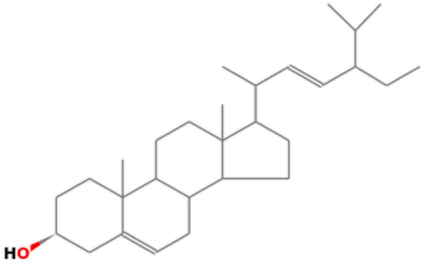
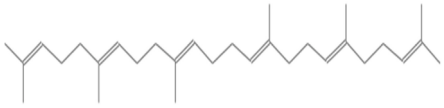
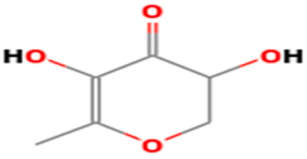
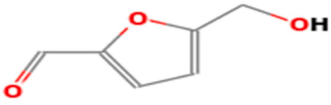
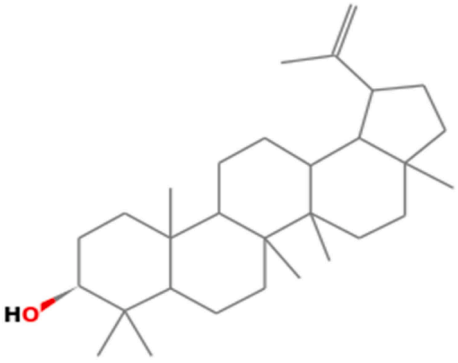
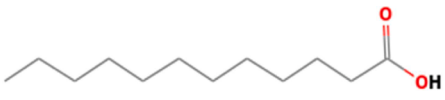


Fig. 2. Chromatogram of methanolic whole plant extract of *Tridex procumbens*.

Table 8
List of compounds present in methanolic whole plant extract of *Tridex procumbens*.

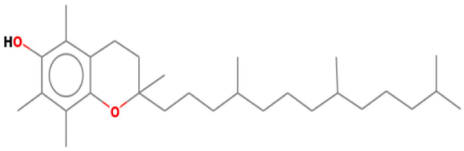
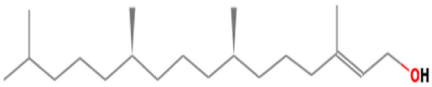
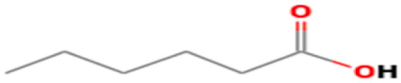
Peak#	R.Time	Area%	Molecular weight	Molecular Formula	Name
1.	8.257	1.11	130	$C_7H_{14}O_2$	1-Butanol, 3-methyl-, acetate
2.	8.652	0.38	144	$C_6H_8O_4$	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-
3.	9.066	0.23	142	$C_8H_{14}O_2$	7-Octenoic acid
4.	9.306	0.14	170	$C_{12}H_{26}$	DODECANE
5.	9.952	0.95	126	$C_6H_6O_3$	5-Hydroxymethylfurfural
6.	10.534	0.13	158	$C_9H_{18}O_2$	Nonanoic acid
7.	11.120	0.32	158	$C_9H_{18}O_2$	2-HEPTANOL, ACETATE
8.	12.188	0.25	206	$C_{13}H_{18}O_2$	1-(3,6,6-TRIMETHYL-1,6,7,7A-TETRAHYDRO-CYCLO
9.	12.525	0.11	188	$C_{13}H_{16}O$	Ethanone, 1-(2,3-dihydro-1,1-dimethyl-1H-inden-4-yl)-
10.	12.734	0.11	150	$C_{10}H_{14}O$	2,2,6-TRIMETHYL-4-METHYLENE-1-OXO-5-CYCLOH
11.	13.333	0.98	144	$C_8H_{16}O_2$	2-Pentanol, 3-methyl-, 2-acetate
12.	13.637	0.14	188	$C_{13}H_{16}O$	3-BUTEN-2-ONE,1-(2,3,6 TRIMETHYLPHENYL)-
13.	13.828	1.41	180	$C_6H_{12}O_6$	D-Allose
14.	14.326	0.32	200	$C_{12}H_{24}O_2$	DODECANOIC ACID
15.	14.587	0.04	222	$C_{12}H_{14}O_4$	1,2-BENZENEDICARBOXYLIC ACID, DIETHYL ESTE
16.	15.495	0.43	194	$C_7H_{14}O_6$	.beta.-D-Glucopyranoside, methyl
17.	15.737	0.12	226	$C_{14}H_{26}O_2$	1-Isobutyl-7,7-dimethyl-octahydro-isobenzofuran-3a-ol
18.	15.797	0.16	224	$C_{13}H_{20}O_3$	3-Buten-2-one,4-(4-hydroxy-2,2,6-trimethyl-7-oxabicyclo
19.	16.004	0.08	210	$C_{13}H_{22}O_2$	2-Cyclohexen-1-one,4-(3-hydroxybutyl)-3,5,5-trimethyl-
20.	16.596	1.85	228	$C_{14}H_{28}O_2$	Tetradecanoic acid
21.	16.750	0.16	224	$C_{14}H_{24}O_2$	2-Pentanone,4-(1,3,3-trimethyl-7-oxabicyclo[4.1.0]hept-2-
22.	16.849	0.49	196	$C_{11}H_{16}O_3$	2(4H)-BENZOFURANONE, 5,6,7,7A-TETRAHYDRO-6-
23.	16.976	0.70	222	$C_{13}H_{18}O_3$	(S,E)-4-Hydroxy-3,5,5-trimethyl-4-(3-oxobut-1-en-1-yl)cy
24.	17.225	0.13	280	$C_{20}H_{40}$	2-Hexadecene, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-
25.	17.297	3.78	278	$C_{20}H_{38}$	Neophytadiene
26.	17.361	0.60	268	$C_{18}H_{36}O$	2-Pentadecanone, 6,10,14-trimethyl-
27.	17.550	0.73	278	$C_{20}H_{38}$	Neophytadiene
28.	17.634	0.16	282	$C_{18}H_{34}O_2$	9-OCTADECENOIC ACID (Z)-
29.	17.746	1.44	278	$C_{20}H_{38}$	Neophytadiene
30.	18.090	0.06	382	$C_{24}H_{30}O_4$	Phthalic acid, 3-ethylphenyl octyl ester
31.	18.149	0.10	268	$C_{18}H_{36}O$	Oxirane, hexadecyl-
32.	18.205	0.40	270	$C_{17}H_{34}O_2$	HEXADECANOIC ACID, METHYL ESTER
33.	18.714	15.37	256	$C_{16}H_{32}O_2$	n-Hexadecanoic acid
34.	19.416	0.44	282	$C_{18}H_{34}O_2$	9-OCTADECENOIC ACID (Z)-
35.	19.667	1.00	280	$C_{18}H_{32}O_2$	13-Hexyloxacyclotridec-10-en-2-one
36.	19.787	0.21	296	$C_{20}H_{40}O$	2-HEXADECEN-1-OL, 3,7,11,15-TETRAMETHYL-,
37.	19.851	0.54	294	$C_{19}H_{34}O_2$	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
38.	19.907	0.16	298	$C_{18}H_{31}$	(9E,12E)-9,12-OCTADECADIENOYL CHLORIDE
39.	20.021	3.39	296	$C_{20}H_{40}O$	Phytol
40.	20.141	0.13	298	$C_{19}H_{38}O_2$	OCTADECANOIC ACID, METHYL ESTER
41.	20.336	2.07	280	$C_{18}H_{32}O_2$	9,12-Octadecadienoic acid (Z,Z)-
42.	20.567	0.83	284	$C_{18}H_{36}O_2$	Octadecanoic acid
43.	21.419	0.38	250	$C_{12}H_{26}O_3S$	Sulfurous acid, isohexyl hexyl ester
44.	21.550	0.71	187	$C_{10}H_{21}NO_2$	Hexanoic acid, 2-dimethylaminoethyl ester
45.	21.649	0.53	312	$C_{19}H_{36}O_3$	9-Octadecenoic acid, 12-hydroxy-, methyl ester, [R-(Z)]-
46.	21.906	0.14	296	$C_{19}H_{36}O_2$	METHYL DIHYDROMALVALATE
47.	22.091	0.31	352	$C_{22}H_{40}O_3$	3-OCTADECYLDIHYDRO-2,5-FURANDIONE #
48.	22.161	0.14	324	$C_{21}H_{40}O_2$	4,8,12,16-Tetramethylheptadecan-4-olide
49.	22.341	0.17	404	$C_{25}H_{40}O_4$	2-[2-Carboxyethyl]-3-methyl-tetrahydrofurano[4,5-a]andros
50.	22.989	0.20	251	$C_{15}H_{25}NO_2$	2-(DIMETHYLAMINO)ETHYL 1-ADAMANTANECARB
51.	23.122	0.27	254	$C_{15}H_{26}O_3$	2,6-DIMETHYL-8-(TETRAHYDRO-2H-PYRAN-2-YLOX
52.	23.289	0.28	274	$C_{16}H_{31}C_lO$	Palmitoyl chloride
53.	23.497	0.95	330	$C_{19}H_{38}O_4$	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl este
54.	24.325	0.53	280	$C_{18}H_{32}O_2$	13-HEXYL-OXA-CYCLOTRIDEC-10-EN-2-ONE
55.	24.577	1.75	313	$C_{17}H_{31}NO_4$	Fumaric acid, 2-dimethylaminoethyl nonyl ester
56.	24.758	2.49	338	$C_{21}H_{38}O_3$	Glycidyl oleate
57.	24.969	3.65	300	$C_{18}H_{33}C_lO$	Oleoyl chloride
58.	25.183	0.24	358	$C_{21}H_{42}O_4$	Octadecanoic acid, 2,3-dihydroxypropyl ester
59.	25.763	0.19	281	$C_{18}H_{35}NO$	9-OCTADECENAMIDE
60.	26.027	1.57	410	$C_{30}H_{50}$	Squalene
61.	26.437	0.36	686	$C_{34}H_{38}O_{15}$	.beta.-d-Glucopyranoside, 5-(acetyloxy)-7-[(acetyloxy)meth
62.	26.630	0.17	462	$C_{29}H_{50}O_4$	.alpha.-Tocospiro A
63.	26.765	3.13	298	$C_{18}H_{34}O_3$	9-Octadecenoic acid, 12-hydroxy-
64.	27.182	28.87	280	$C_{18}H_{32}O_2$	13-HEXYL-OXA-CYCLOTRIDEC-10-EN-2-ONE
65.	28.219	0.28	368	$C_{24}H_{48}O_2$	Hexanoic acid, octadecyl ester
66.	28.370	0.27	266	$C_{15}H_{22}O_4$	1,5,5-TRIMETHYL-6-[(1E)-3-OXO-1-BUTENYL]-7-OXA
67.	29.677	0.24	454	$C_{31}H_{50}O_2$	Stigmasta-5,22-dien-3-ol, acetate, (3.beta.)-
68.	30.599	0.40	430	$C_{29}H_{50}O_2$	Vitamin E
69.	32.774	0.84	400	$C_{28}H_{48}O$	Ergost-5-en-3-ol, (3.beta.)-
70.	33.377	2.56	412	$C_{29}H_{48}O$	Stigmasterol
71.	34.949	2.62	414	$C_{29}H_{50}O$	STIGMAST-5-EN-3-OL, (3.BETA.,24S)-
72.	35.208	0.70	424	$C_{30}H_{48}O$	.beta.-Amyrone
73.	35.351	0.28	412	$C_{29}H_{48}O$	Fucosterol
74.	36.061	0.94	426	$C_{30}H_{50}O$	.beta.-Amyrin
75.	36.552	0.69	424	$C_30H_{48}O$	Lup-20(29)-en-3-one
76.	37.444	0.98	426	$C_{30}H_{50}O$	Lupeol

Table 9
list of important phytoconstituents identified in investigated plant sample and their known biological activities.

S. No.	Compound name	Chemical class	Chemical Structure	Biological Activities
1.	n-Hexadecanoic acid	Fatty acid		Anti-inflammatory, antioxidant, anti-androgenic, hypocholestrolemic. Kumar et al., (2010); Aparna et al., (2012)
2.	Hexadecanoic Acid, Methyl ester	Fatty acid ester		Antioxidant, antimicrobial hypocholestrolemic, nematicide hemolytic Duke's Phytochemical and Ethanobotanical Databases, (2016); Aleryani, (2005)
3.	Octadecanoic acid	Fatty acid		Octadecanoic acid; Antimicrobial activity Duke's Phytochemical and Ethanobotanical Databases, (2016)
4.	Stigmasterol	Phytosterol		Anti-osteoarthitis, cholesterol lowering activity, Anticancerous, Thyroid inhibiting, antiperoxidative, hypoglycemic. Chen et al., (2012); Ghosh et al., (2012); Panda et al., (2009).
6.	Squalene	Triterpene		Anti-inflammatory, cytotoxic, anticancer, antimicrobial Spanova and Daum, (2011)
7.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Flavonoids		Treat osteoporosis, diabetes, and cardiovascular disease. Chen et al., (2021), Yu et al., (2013)
8.	5 Hydroxymethylfurfural	Furans		antimicrobial properties. Zhang et al (2009)
9.	Lupeol	Triterpenoid		antimicrobial activity, Antioxidant, Hepatoprotective and anticancer properties. Siddique and Saleem (2011); Ghani, U (2011); Geetha and Varalakshmi (2001); Prasad and Kalra (2010).
10.	Dodecanoic acid	Saturated fatty acids		Antibacterial properties Nakatsuji et al (2009).

(*continued on next page*)

Table 9 (*continued*)

S. No.	Compound name	Chemical class	Chemical Structure	Biological Activities
11.	Vitamin E (α-Tocopherol)	Tocopherols		Antioxidant, antimicrobial, Cardioprotective Properties. Serafini et al (2002); Traber (2007).
12.	Neophytadiene	Diterpene hydrocarbon		Antibacterial activity. Balouiri et al (2016).
13.	Phytol	Terpenoids		Antimicrobial and antiparasitic properties. Nogueira et al (2017).
14.	Hexanoic Acid	Carboxylic acids		Antimicrobial activity. Desbois and Smith (2010).

fresh extracts of chloroform and petroleum ether root extracts of *E. echinatus* showed the highest zone of inhibition against *P. putida, P. syringae, S. aureus and E. aerogenes* while leaves, flowers and stem extracts showed moderate activities against all seven bacterial strains. In Table 2: *E. echinatus* root and flowers dry extracts have maximum zone of inhibition against *E. aerogenes, E. coli* and *P. syringae*. Petroleum ether, alcoholic, and chloroform extracts of leaves and stem shows moderate zone of inhibition against all seven bacterial strains while aqueous extracts of all plant part have minimum zone of inhibition. In Table 3: fresh root, flower and leaves of *Tridex procumbens* have shown significant zone of inhibition against all seven bacterial strains while flower extracts have maximum inhibition zone against *A. tumefaciens*. In Table 4: dry leaves and root have highest antimicrobial potential against *E. aerogenes, S. aureus, B. subtilis* and *P. syringae* while whole plant showed the significant activity against all seven bacterial strains. According to Hymete et al. (2005), Leaf extracts of *E. longisetus* and *E. ellenbeckii* showed strong inhibitory activity against *Staphylococcus aureus* while stem extract of *E. longisetus* showed strong inhibitory activity against cultures of *Staphylococcus aureus*. The flower extract of *E. ellenbeckii* showed strong inhibitory activity against *Candida albicans*. Root and flower extracts of the plants showed lethal activity against earthworms. While aqueous extracts of *T. procumbens* has antibacterial activity against *Trypanosoma brucei* and also possesses wound-healing properties (Koram et al., 2014, Agyare et al., 2016). *Citrus limon* exhibits antibacterial activity against several pathogenic strains of bacteria (Bissa and Bohra 2022). A study by Singh et al. (2021) evaluated the antibacterial properties of extracts from *Lawsonia* inermis and *Adhatoda vasica* against *E. coli, E. aerogenes, S. typhi*, and *A. tumefaciens*.

Preliminary Phytochemical Results

In *Echinops echinatus* and *Tridex procumbens* various phytochemical present such as alkaloids, glycosides, flavonoids, saponins, tannins and terpenoids are present in leaves, stem, flower, immature pods and gum (Figs. 1, 2 and Tables 5-9).

Conclusion

It can be concluded from the present study that *Echinops echinatus* and *Tridex procumbens* are ethnomedicinally important plant used to treat various diseases and both plants have antimicrobial potential against *Escherichia coli, Enterobacter aerogenes, Agrobacterium tumefaciens, Staphylococcus aureus, Bacillus subtilis Pseudomonas putida* and *Pseudomonas syringae*. The extract contains the active phytoconstituents including alkaloids, glycosides, flavonoids, saponins, tannins and terpenoids. The GCMS analysis concludes several compounds responsible for their antimicrobial properties.

Author contribution

NS and NC prepared the manuscript; SB did corrections in the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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