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Bioactive potential of *Distimake dissectus* leaf: GC–MS analysis and *in-vitro* bioassays for antioxidant, antimicrobial, and anti-inflammatory properties

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Key words: GC-MS analysis, Antioxidant, Antimicrobial, Anti-inflammation, *Distimake Dissectus* and Convolvulaceae

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Abstract

Distimake dissectus (Jacq.) A.R. Simões & Staples. is belonging to the family Convolvulaceae. It is commonly called as "alamo vine" is a perennial herb. The current study investigates the GC-MS characterization, antioxidant, antimicrobial, and anti-inflammatory properties of D. *dissectus*. The methanolic leaf extract included 53 different phytochemicals, as determined by the GC-MS analysis.The DPPH radical scavenging assay revealed the IC_{50} values of methanol extract 160.03±0.857, hexane extract 145.61±0.62, and ascorbic acid 24.12±0.313 respectively. The anti-bacterial activity was studied by the Agar well-diffusion method with four concentrations (25 µl, 50 µl,75 µl and 100 µl). The leaf extracts showed remarkable antibacterial activities, the maximum zone of inhibition observed in methanol extract compared to hexane extract for each bacterium was as follows., *S. aureus* (100µg/18mm), *E. coli* (100µg/18mm), *S. pneumonia*, (100µg/13mm), *P. aeruginosa* (100µg/14mm), the MIC was recorded its active at concentration of 200 µg/ml in *S. aureus* and *E. coli*. The anti-fungal activity was tested by well diffusion method, the methanol extract showed *C. albicans* (100µg/16mm) and hexane (100µg/14mm). The Griess test, which measures the anti-inflammatory response, was employed to assess the substances that inhibit the production of NO, at a concentration of 100 µg/ml, the methanol extract exhibited a NO inhibition of 49.04±0.457. The current study's findings will pave the way for the creation of herbal remedies using. The findings of the current study on *D. dissectus* may pave the way for the development of herbal remedies for a variety of illnesses, potentially resulting in the creation of innovative therapeutics.

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Introduction

Therapeutic products derived from plants have been used since prehistoric times. Many commonly employed current medical treatments originate from plants, either directly or indirectly. In certain cases, phytomedicines—compounds produced from plants—are more effective than drugs made from synthetic ingredients and are also safer, less expensive, and more affordable for patients to obtain (Gajula *et al.,* 2024).

Modern synthetic and chemical medications are frequently investigated cautiously due to their negative effects (Philomena 2011), In contrast, traditional herbal remedies are becoming more popular due to their natural ingredients, environmental friendliness, and lack of adverse effects (Sahoo *et al.,* 2013).Therefore, despite all of the advantages of contemporary synthetic supplements, human beings continue to choose natural medications derived from plants over those made from chemicals(Yuan *et al.,* 2016), The medicinal plants has unique properties to both treat and cure a wide range of human problems due to the presence of important phytoconstituents in diverse portions of the plants (Anand *et al.,* 2019; Semwal *et al.,* 2019; Yuan *et al.,* 2016). In India, for centuries, diverse components of medicinal plants (about 80,000 species) have been used as traditional remedies in different Indian medical systems to treat a wide range of ailments (Pandey *et al.,* 2013), Currently, around 25% of the active components found in medicinal plants, which have been used as prescription medications, have been identified (Pandey *et al.,* 2008). According to some sources, there are more than 25,000 plant-based formulations in the Indian systems of folk and traditional medicine. Around 1.5 million practitioners administer these formulations for preventative, persuasive, and therapeutic purposes (Sreenivasa *et al.,* 2020). Medicinal plants include diverse bioactive chemicals that possess pharmacological activities such as antibacterial, antifungal, anticancer, anti-inflammatory, and antioxidant effects (Yuan *et al.,* 2016, Anand *et al.,* 2019, Malongane *et al.,* 2017). The potential of these substances should be explored in order to determine whether they are suitable for treating various illnesses (Anand *et al.,* 2019, Pandey *et al.,* 2008). Plant-based treatments frequently originate from raw plant extracts that include a diverse combination of several phytochemicals (Pandey *et al.,* 2018). Phytochemicals possess distinctive and intricate structures, and are used in the treatment of both chronic and communicable illnesses (Sahoo *et al.,* 2013; Pandey *et al.,* 2008). Although many plant species contain a vast reservoir of bioactive secondary metabolites, only a small percentage of them have been investigated and proven to be valuable sources of bioactive compounds. The development of appropriate screening procedures is essential for the identification of novel compounds and for ensuring quality control (Keskes *et al.,* 2017). The process of extracting and analyzing a wide range of bioactive components from different medicinal plants has resulted in the development of very potent medications (Yadav *et al.,*2017), The preliminary evaluation of medicinal plants using spectrometric and chromatographic techniques yields fundamental data on their chemical composition and pharmacological properties, facilitating the identification of biologically active plants (Juszczak *et al.,* 2019).In the last few years, gas chromatography mass spectrometry (GC-MS) has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non-plant species (Robertson 2005; Fernie *et al.,* 2004; Kell *et al.,* 2005), Gas chromatography – Mass spectrometry is an important technique that has been adapted to evaluate different phytoconstituents present in various plant extracts with their structures.

This technique has superior separation potency that leads to produce a high accuracy and precision of chemical fingerprint. Moreover, quantitative data along with the coupled mass spectral database can be given by GC-MS that is of tremendous value for achieving the correlation between bioactive compounds and their applications in pharmacology (Kumar *et al.,* 2019).

Free radicals are essential in the progression of tissue destruction during pathogenic occurrences. Antioxidants are chemical substances that possess the capacity to neutralize free radicals, therefore safeguarding the human body against a range of ailments. Plants are abundant in antioxidants, which are molecules that possess secondary metabolites like phenolic and flavonoid chemicals. These compounds have antioxidant capabilities and may effectively reduce oxidation and bind to metals (Karimi and Jaafar, 2011). Antioxidants are chemical compounds extremely useful to humans, which has the ability to reduce free radicals and/or to decrease their rate of production and lipid peroxidation in human bodies that cause various human diseases and aging (Galati and Brien, 2004). Plants provide us with rich sources of natural antioxidants (Biswas *et al.,* 2005). About 80% of the world population depends on plant-based medicines as a source of primary health care in rural areas of both developing and developed countries, where modern medicines are mainly used the plant extracts have always been used as an alternative source of antimicrobial compounds. The recent spread of multi-drug-resistant bacteria and their increased treatment costs necessitated the study of alternative, cheap sources (Seth, 2004). Infectious diseases are a major public health problem in the world. They are one of the leading causes of death. The increased use of antibiotics in the past few years, both in appropriate and inappropriate ways, has led to increased antibiotic resistance. Bacteria are able to resist various antibiotics through horizontal gene transfer or mutation (Reygaert, 2018). It is expected that by 2050, 10 million lives will be lost per year, and a cumulative 100 trillion USD of economic output will be at risk due to the rise of drug-resistant infections if we do not find proactive solutions now to slow down the rise of drug resistance (O'Neill, 2023).The increasing failure of antimicrobials and antibiotic resistance shown by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activities (Iwu *et al.,* 1999). Inflammation is often defined as an intricate biological reaction of blood vessel tissues to detrimental stimuli. In addition, inflammation causes pain and is characterized by elevated protein denaturation, increased vascular permeability, and membrane modification, among other factors (Ferrero-Millani *et al.,* 2007). Inflammation is the body's reaction to neutralize or eliminate foreign substances or organisms, eliminate irritants, and prepare the tissue for healing. This process is expedited by the release of chemical mediators from damaged cells or tissues and migratory cells (Chandra *et al.,* 2012). There are two classifications of inflammation: acute and chronic. Acute inflammation is the initial response of the body to harmful stimuli, and is achieved by the progressive movement of plasma and leukocyte-like constituents from the blood, into the injured tissues/locations. Chronic inflammation leads to a progressive shift in the type of cells present at the site of inflammation, and is characterized by simultaneous breakdown and healing of the tissue from the inflammatory process (Leela Prakash and Dass, 2011). Therefore, the search for natural sources and phytochemicals with anti-inflammatory activity has greatly increased in recent years.

Distimake dissectus (Jacq.) A.R. Simões & Staples commonly called as "alamo vine" belonging to family Convolvulaceae (Austin *et al.,* 2007). is a perennial herb growing in a human distributed region (Roadside, hedges). The leaves are alternate to10 cm in length and palmately divided with 7-9 lobes, the lobe margins are sinuate to dentate with an acuminate apex. The Geographical Distribution is Native of Tropical and Subtropical America whereas distributed globally, India, Sri Lanka, Myanmar, China, Pakistan Indonesia, Philippine, Australia, French Polynesia, Papua, New Guinea, Solomon Islands, Samoa, Tonga, and Seychelles (Staples *et al.,* 2010). In India, the plant is observed in various regions but presence in a area like Kerala, Uttar Pradesh, Maharashtra, Andhra Pradesh and Telangana have been reported (Sasidharan *et al.,* 2020; Srivastava *et al.,* 2017). In folk medicine leaves and its juice are used as sedative and for urinary tract infections. The plant is also a remedy for skin diseases. Boiled tubers are used traditionally against

chest problems, inflammation, urinary tract infections, snake bite, sprains and scabies (Morton 1981; Mansur *et al.,* 2001; Hawthorne *et al.,* 2004; DeFilipps *et al.,* 2004; Ved *et al.,* 2002). Anti-elastase β activity, antimicrobial and antibiofilm activity (Jawad Ahmad *et al.,* 2002); anti-asthmatic, anti-hyperglycemic, anti-nociceptive, anti-inflammatory, immunomodulatory and anticarcinogenic (Qadir *et al.,* 2017); calystegine B2 (Schimming *et al.,* 2005); antidiabetic (Gaikwad *et al.,* 2014), antibiofilm and antielastase activity (Luciardi *et al.,* 2016); Antimicrobial activity (Jasim *et al.,* 2018; Theng *et al.,* 2015; Shikha *et al.,* 2015); Antifungal activity (Joshi *et al.,* 2015). The GC-MS of *M. dissectus* leaf showed 40 different compounds by (Putten, 1979; Ridhi Joshi *et al.,* 2018; María *et al.,* 2016). GC-MS, antimicrobial and antioxidant by (Abdel Karim *et al.,* 2021). In Vitro Antiarthritic Activity of synthesized silver nano particles (Rucha Pancham *et al.,* 2020), cold infusion was remedy for giddiness and is given as a treatment for chest complaints in children (Dalziel, 1937); chest problems, inflammation, emollients and sedatives reported by (Roig and Mesa, 1945), urinary infections (Morton, 1981), snakebite and intoxication in Africa (Mansur, 2001), In 2022, the global herbal medicines market was worth USD 170 billion, and the expectation is that this market has the potential to reach USD 600 billion by 2033, with a compound annual growth rate (CAGR) of 15% from 2023 to 2033 (Newsmantraa, 2023).

Material and methods

Collection and authentication of plant material

The leaves of *Distimake dissectus* were collected from Anantha Giri Hills is located in Vikarabad district, of Telangana State India during August / September in the year 2022.

The plant was authenticated by Botanical Survey of India, Deccan Regional Centre, Hyderabad, Telangana (accession number- BSI/DRC/2023-24/Identification/436), and the specimen was submitted to the Herbarium, Hyderabadensis, Department of Botany, O.U, Hyderabad, Telangana India.

Drying

The leaves were fragmented into pieces of (0.5—1.5×1×0.2 cm3) utilizing scissors and knives after being sanitized. The newspapers were used to shade dry them out over a duration of 10 days. Subsequently, the fruit pieces underwent dehydration in a high-temperature oven set at 40 °C for one hour just before to commencing the extraction procedure in order to eradicate any remaining moisture.

Preparation of crude extracts with Successive Extraction Using Soxhlet Apparatus

To prepare the extracts of *D. dissectus* leaves, fresh leaves were obtained and washed thoroughly with flowing water to eliminate any dirt or debris. The leaves were extracted using various solvents including hexane, chloroform, ethyl acetate and methanol. Subsequently, the leaves were rinsed again under flowing distilled water to remove any residual contaminants. Dried leaf powder was obtained through a mechanical grinder and sieved through to get the powder of uniform size. The fruit powder was extracted successively in the Soxhlet apparatus with hexane at 60° C, ethyl acetate at 77° C, chloroform at 61° C, and methanol at 65° C. The extraction temperatures were adjusted to boiling points of solvent to allow a faster rate of cycling of fresh solvent. Six hours of duration was allocated to each solvent for hot continuous and successive extraction. The extracts were filtered using Whatman No.1 filter paper to obtain particle free extract. The residue was reextracted twice by solvent and filtered. All extracts were filtered followed by evaporation to desire volume by a rotatory evaporator. The obtained extract was stored in desiccator for GC-MS for analysis of different compounds (Raman, 2006; Anonymous, 2015; Gajula *et al.,* 2002; Kamalakar *et al.,* 2014).

Antioxidant activity studies

2-Diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Activity

The free radical scavenging activity of the extracts were analyzed by the 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) method according to Baliyan *et al.,* (2022). This method is based on the reduction of

DPPH in methanol solution in the presence of hydrogen donating antioxidant due to the formation of non-radical from DPPH- H, the transformation results in the formation of color change from purple to yellow. In this assay, varying concentrations (50,75,100,150 and 200µg/ml) of compound in 1 ml of methanol solution of DPPH (0.2 mM). There is a need to prepare fresh DPPH 0.2 mM and set OD value to 0.8. If the O.D is less than 0.8 add DPPH or more than 0.8 add methanol. The mixture was thoroughly mixed and incubated for 30 min. The optical density of the solution was then measured at 517 nm using spectrophotometer. Percent inhibition of antioxidant activity was calculated by using the following formula and readings of test sample are compared with the positive control of ascorbic acid (Vitamin C).

$$\text{Percentage of inhibition of DPPH} = (\text{Control OD} - \text{Test OD}) / \text{Control OD}) \times 100.$$

Total antioxidant capacity (phosphomolybdenum assay)

To determine the Total Antioxidant Capacity (TAC) of the plant fractions, the phosphomolybdenum method was described by (Hussen and Endalew, 2023). In brief, 0.3 mL of each solvent fraction and standard drug (at concentrations ranging from 25 µg/mL to 400 µg/mL) were mixed with 3 mL of a reagent solution consisting of 0.6 M sulphuric acid, 4 mM ammonium molybdate, and 28 mM sodium phosphate in test tubes. The test tubes were covered and incubated in a water bath at 95°C for 95 minutes. After cooling to room temperature, the absorbance was measured at 695 nm. A mixture containing distilled water instead of the samples was used as a control. Standard drugs such as ascorbic acid and gallic acid were also used. Higher absorbance values indicate higher total antioxidant potential.

$$\text{The percentage inhibition (\%)} = \left\{ \frac{(\text{absorbance of sample} - \text{absorbance of control})}{(\text{absorbance of sample})} \right\} \times 100$$

Gas Chromatographic Mass Spectroscopy (GC-MS) analysis

GC-MS analyses of methanol extract were performed using a Shimadzu QP2010 Gas Chromatography–Mass spectroscopy. It employed a fused silica column packed with Elite -5 ms [5% Diphenyl 95% Dimethyl poly siloxane, 30 mm × 0.25 mm × 0.25 µm df] and the components were separated using helium as carrier gas at a constant flow of 1ml / min.

The 2 µl sample extract injected in to the instrument. It was detected by the turbo gold mass detector with aid of Turbo mass 5.2 software. During the GC Process the oven was maintained at temperature of 110°C with 2 min holding. The injector temperature was set at 250°C. The inlet line temperature was 200 °C and source temperature was 200°C. Mass spectra were taken at 70 eV, a scan period of 0.5 S and fragment from 45 - 450 Da. The MS detection was completed in 35 min. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute standard and technology (NIST and WILEY) having more than 62,000 patterns. The spectrum of unknown components stored in the NIST and WILEY library (Al Abboud *et al.,* 2023).

Antibacterial activity

(Bhandari *et al.,* 2023; Meri Amerikova *et al.,* 2019).

Bacterial Strains

The bacterial strains Gram positive of *Staphylococcus aureus* (ATCC 25923), *Streptococcus pneumonia* (ATCC 33400), and Gram negative of Pseudomonas *aeruginosa* (ATCC 27853), *E. coli* (ATCC 25922) used in the study were obtained from ATCC.

Media preparation for anti-bacterial activity

Nutrient agar media

Nutrient Agar we procured from commercially and weighed 28.0 gms of powder dissolved in 1000 ml distilled water and mix thoroughly. Sterilized the dissolved nutrient agar in autoclave at 121° C for 15 mins and used the media for plate preparation to study the anti-bacterial activity.

Nutrient broth

Nutrient Brothwe procured from commercially and weighed 1.3 gms of powder dissolved in 100 ml

distilled water and mix thoroughly. Sterilized the dissolved nutrient broth in autoclave at 121° C for 15 mins and used the broth for inoculum preparation.

Preparation of stock solution

The stock culture of each organism was prepared by taking two nutrient agar slants and sub culturing each confirmed test organism aseptically. One set slant was kept as stock culture and another as working set.

The cultures of bacteria in their appropriate agar slants were stored at 4°C and used as stock cultures. One counter glycerol stock was also maintained at 20° C temperature.

Inoculum preparation

The selected bacterial pathogens were inoculated into nutrient broth and incubated at 37°C for 24 hours and the suspensions were checked to provide approximately 10-5 CFU/ml.

Antibacterial activity

Anti-bacterial activity chemical compoundsstudied by the Agar well-diffusion method with four concentrations (25, 50,75 and 100 µl) were tested against different bacterial pathogens such as of *Staphylococcus aureus, Streptococcus pneumonia, Pseudomonas aeruginosa* and *E. coli*. The plates were incubated at 37°C for 18-24 hrs and end of the experiment the diameter of the inhibition zone (mm) was measured and the activity index was also calculated. The readings were taken in three different fixed directions and the average values were recorded.

Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC), is the lowest concentration of an anti-microbial growth that will inhibit the visible growth of a microorganism after overnight incubation.

Compound preparation

Compounds were weighed individually 1mg and dissolved in methanol for final stock concentration as 1mg/ml. As same sample, standard amoxicillin also prepared.

Culture preparation

Loop of culture was inoculated in 3 ml of nutrient broth and incubate 37 ° c for overnight in shaking incubator.

Inoculum preparation

From overnight grown culture, 20 µl of culture was taken and inoculated in 1.5 ml of nutrient broth and added different concentration of compound and incubated at 37 ° c for overnight in an incubator.

Anti-fungal activity

(Sumaiya Naeema Hawar *et al.,* 2023)

Fungal Strains

The fungal strains *Candida albicans* (MTCC 183) used in the study was obtained from Microbial type culture collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh.

Sabouraud dextrose agar (SDA)

Sabouraud dextrose agar procured commercially and weighed 32.5 gms of powder dissolved in 500 ml distilled water and mix thoroughly. Sterilized the dissolved SDA in autoclave at 121° C for 15 mins and used the SDA for the plate preparation to study the anti-fungal activity.

Antifungal activity

Anti-fungal activity was tested by well diffusion method w. The prepared SDA culture plates were spreader with *Candida albicans* fungus using spread plate method. The plates were incubated at 37+2°C for 48 hrs for fungal activity. After 48 hrs, the plates were observed for zone formation around the well and the zone of inhibition (mm) was measured.

Anti-inflammatory activity

(Mustafa, 2023; Yilma Hunde Gonfa *et al.,* 2023).

Cell Culture

Murine macrophage RAW 264.7 cells were maintained in Dulbecco's modified eagle media (DMEM) supplemented with 10% fetal bovine serum (FBS) and antibiotics (50 U/ml of penicillin and 50

mg/mL streptomycin, Sigma-Aldrich) at 37°C in a humidified atmosphere containing 5% CO2.

Determination of anti-inflammatory activity

To study the inflammatory response, Griess assay was performed to evaluate the compounds inhibiting the NO production. For this RAW264.7 macrophage cells were plated at a density of 2×10^5 cells/well in 96-well culture plate and incubated at 37°C for 3-4 hrs. Plated cells were treated with lipopolysaccharides (LPS, 1 µg/mL) for stimulation of nitrite oxide production. Cells were further incubated at 37°C, 5% CO2 for 20 hrs. Griess reagent (1% sulfanilamide/ 0.1% N-(1-naphtyl) ethylene diamine dihydrochloride in 2.5% H3 PO4) was mixed with an equal part of cell culture medium of control and samples treated RAW 264.7 cells. The absorbance of the mixture was determined at 540 nm by a micro plate reader. The cells were stimulated with LPS for 1 hr before the treatment with Sample dissolved in DMSO at different non-cytotoxic concentrations (5–100µg/ml). The percentage NO inhibition was determined according to the formula below.

$$\frac{\text{NO level of control - NO level of compounds treated cells}}{\text{NO level of control cells}} \times 100$$

Results

Morphological description

Distimake dissectus (Jacq.) A.R.Simões & Staples, is commonly called as "alamo vine" belonging to family Convolvulaceae. It is a broad-leafed, perennial herbaceous climber that reaches a height of approximately 4 m on hairy, counterclockwise twining stems. The leaves are simple, arranged in a spiral, and have 2-4 cm long, extremely hairy stems.

Typically, the leaf blade measures 4-8 cm in length and 6-12 cm in width. It is divided into 3-7 segments, each deeply 5-7 lobed, and palmately lobed.

Table 1. GC-MS analysis.

Pea	R.Time	Area	Area%	Height	A/H	Base m/z	Name
1	1.040	454148	2.20	394263	1.15	44.05	Carbon dioxide
2	1.090	9612105	46.61	4413959	2.18	47.10	Ethane, 1-chloro-1-fluoro-
3	1.140	4840592	23.47	5320233	0.91	45.10	Ethanol
4	1.186	1053792	5.11	822050	1.28	45.05	Isopropyl Alcohol
5	12.492	90306	0.44	26819	3.37	161.15	Germacrene D
6	12.662	162892	0.79	53609	3.04	121.25	Germacrene B
7	12.819	175195	0.85	47460	3.69	68.05	8-Isopropenyl-1,5-dimethyl-cyclodeca-1,5-diene
8	27.210	71119	0.34	25440	2.80	207.05	Silane, trimethyl[5-methyl-2-(1-methylethyl)phenoxy]-
9	28.240	66088	0.32	19969	3.31	207.05	Cyclotrisiloxane, hexamethyl-
10	28.290	175434	0.85	20997	8.36	207.05	Cyclotetrasiloxane, octamethyl-
11	28.440	30797	0.15	27562	1.12	281.10	2,4-Imidazolidinedione, 3-methyl-5,5-diphenyl-1-(trimethylsilyl)-
12	28.460	20690	0.10	15225	1.36	281.15	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane
13	28.480	78807	0.38	33453	2.36	207.10	Silane, 1,4-phenylenebis[trimethyl-
14	28.530	64485	0.31	27912	2.31	44.05	Tartronic acid, 4-(dimethylethylsilyl)phenyl-, dimethyl ester
15	28.695	130977	0.64	11941	10.97	44.05	N-(10,11-Dihydro-5H-dibenzo[b,f]azepin-5-yl)malonamic acid
16	29.110	53206	0.26	33938	1.57	207.10	Silicic acid, diethyl bis(trimethylsilyl) ester
17	29.430	57441	0.28	32091	1.79	207.05	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene
18	29.526	45572	0.22	39018	1.17	207.05	Tetrasiloxane, 1,1,3,3,5,5,7,7-octamethyl-
19	29.600	31075	0.15	13494	2.30	281.10	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane
20	29.785	24864	0.12	29150	0.85	207.10	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-
21	29.909	311882	1.51	58621	5.32	207.05	1,2-Dihydro-2,4-diphenyl-quinazoline
22	30.060	194912	0.95	66282	2.94	282.15	[2-(5-Hydroxypent-2-enyl)-3-oxocyclopentyl]thioacetic acid, S-t-butyl ester
23	30.301	57341	0.28	43205	1.33	207.05	Silicic acid, diethyl bis(trimethylsilyl) ester
24	30.509	133655	0.65	48633	2.75	281.15	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane
25	30.641	157847	0.77	48693	3.24	207.05	1,3,5,7-Tetraethyl-1-butoxycyclotetrasiloxane
26	30.715	93767	0.45	29170	3.21	281.10	1-Pentene, 4,4-dimethyl-1,3-diphenyl-1-(trimethylsilyloxy)-
27	30.726	24085	0.12	46183	0.52	281.15	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane

28	30.790	156200	0.76	64872	2.41	207.10	Silicic acid, diethyl bis(trimethylsilyl) ester
29	30.868	24877	0.12	41083	0.61	281.10	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-
30	31.050	25525	0.12	36044	0.71	207.10	Cyclotrisiloxane, hexamethyl-
31	31.070	26847	0.13	25305	1.06	207.05	2-Chloroaniline-5-sulfonic acid
32	31.099	30689	0.15	41744	0.74	207.10	Propiophenone, 2'-(trimethylsiloxy)-
33	31.145	103133	0.50	43970	2.35	207.10	3,3-Dibutoxy-1,1,1,5,5,5-hexamethyltrisiloxane
34	31.214	76202	0.37	48539	1.57	207.05	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-
35	31.300	138980	0.67	47581	2.92	281.10	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane
36	31.350	77101	0.37	33768	2.28	207.05	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl-
37	31.438	166208	0.81	50585	3.29	96.05	(E)-4'-Methyl-2-methoxymethoxy-stilbene
38	31.515	133190	0.65	56238	2.37	281.10	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-
39	31.525	34582	0.17	63273	0.55	281.15	Pentasiloxane, 1,1,3,3,5,5,7,7,9,9-decamethyl-
40	31.535	42630	0.21	87937	0.48	281.10	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane
41	31.564	223259	1.08	125841	1.77	207.05	Cyclopenteno[4.3-b]tetrahydrofuran, 3-[(4-methyl-5-oxo-3-phenylthio)tetrahydrofuran-2-ylo
42	31.610	205607	1.00	83356	2.47	207.10	3-Isopropoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane
43	31.650	103270	0.50	70968	1.46	281.15	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane
44	31.670	50969	0.25	42925	1.19	281.10	Cyclotetrasiloxane, octamethyl-
45	32.025	165133	0.80	28536	5.79	281.15	Cyclotetrasiloxane, octamethyl-
46	32.340	77091	0.37	47792	1.61	207.05	Silicic acid, diethyl bis(trimethylsilyl) ester
47	32.385	89834	0.44	58271	1.54	281.10	3-Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy)trisiloxane
48	32.445	43877	0.21	30996	1.42	281.15	Cyclooctasiloxane, hexadecamethyl-
49	32.475	70441	0.34	41002	1.72	281.15	Haloxazolam
50	32.515	44042	0.21	63139	0.70	281.15	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-
51	32.543	47819	0.23	46781	1.02	207.05	Pentasiloxane, 1,1,3,3,5,5,7,7,9,9-decamethyl-
52	32.609	232381	1.13	60990	3.81	281.10	Cyclotetrasiloxane, octamethyl-
53	32.844	21632	0.10	35552	0.61	207.10	Silicic acid, diethyl bis(trimethylsilyl) ester

The flowers are fairly irregular and mostly white with hints of crimson or purple. Five sepals are free of cost. The flower tube has five connected petals forming a funnel shape, and it is 3.5–4.5 cm long. Five stamens are also present. The fruit is a dehiscent, five-valved capsule that is globular, papery, smooth, and non-fleshy. Its length can reach about 2 cm, and its diameter is typically comparable but somewhat greater. It breaks open to expose a tiny seed container with a papery surface that is inside a shape resembling the fruit. There are two black seeds inside, divided by a somewhat rigid wall. Flowering &Fruiting**:** February, April to July, and September (Fig.1A-D).

Table 2. DPPH Scavenging Activity of Ascorbic acid.

Test Compound	Concentration (µg/ml)	OD	% Inhibition	SD	IC_{50}
Ascorbic acid	10	0.326	38.37	0.213	24.12±0.313
	25	0.269	49.14	0.245	
	50	0.142	73.15	0.378	
	75	0.118	77.69	0.461	
	100	0.068	87.14	0.842	
	Control	0.529	0	0	

The methanol extract of the *D. dissectus* leaf underwent gas chromatography and mass spectrometry analysis using a SHIMDZU QP-2020 Gas – Chromatography - Mass Spectrometry instrument. The findings of the spectral comparison between unknown and known chemical substances are shown in Table 8. The data depicts 53 peaks representing the substances that were identified bioactive compounds, which have been determined based on their retention time (RT), molecular formula (MF), molecular structure (MS), molecular weight (MW), and concentration (peak area%).

Table 3. DPPH Scavenging Activity of Methanolic Extracts.

Test Compound	Concentration (µg/ml)	OD	% Inhibition	SD	IC50
Methanolic MD extract	50	0.456	13.79	0.208	160.03±0.857
	75	0.407	23.06	0.234	
	100	0.337	36.29	0.346	
	150	0.272	48.58	0.389	
	200	0.212	59.92	0.522	

The analysis revealed that the major component in the methanol extract was 1-Chlorofluoroethane (C_2H_4ClF) with a molecular weight of 82. It accounted for 46.61% of the peak area. Ethanol (C_2H_6O) with a molecular weight of 46 had the second-highest peak area at 23.47% and exhibited antiseptic activity. Isopropyl alcohol (C_3H_8O) with a molecular weight of 46 had a peak area of 5.11%. Germacrene B ($C_{15}H_{24}$) with a peak area of 0.79% showed antifungal activity.

The lowest peak area observed was 0.70% and belonged to Ethoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy) trisiloxane ($C_{11}H_{32}O_4Si_4$) with a molecular weight of 340. The bioactive molecules that remain, together with their corresponding, are shown in Table 1.

Anti-Oxidant Study of Ascorbic Acid Using DPPH Assay

In accordance with the findings of ebrahimzadeh and bahramian (2009), the 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) technique was used in order to evaluate the extracts' capacity to reduce the amount of free radicals that were present. Following that, a spectrophotometer was used to determine the optical density of the solution at a wavelength of 517 nm.

Table 4. DPPH Scavenging Activity of Hexane Extract.

Test Compound	Concentration (µg/ml)	OD	% Inhibition	SD	IC50
Hexane MD extract	50	0.487	7.93	0.231	145.61±0.628
	75	0.421	20.41	0.278	
	100	0.344	34.97	0.352	
	150	0.273	48.39	0.397	
	200	0.229	56.71	0.504	

Table 2 provides the information necessary to estimate the percentage of free radicals that are inhibited by ascorbic acid at different doses (10, 25, 50, 75, and 100 µg/ml). When doing the DPPH test for scavenging free radicals, ascorbic acid is used as the positive control throughout the experiment. Using the IC_{50} graph, which can be seen in Figure...., the value of 24.12±0.313 µg/ml is derived for the IC_{50}. At different doses of ascorbic acid (10, 25, 50, 75, and 100 µg/ml), the percentage of inhibition was found to be 38.37%, 49.14%, 77.69%, and 87.14%, respectively.

The IC_{50} value above 49% of ascorbic acid shows very strong inhibition of the free radicals.

Table 5. Anti-bacterial activity of the methanolic extract of plant leaves against pathogenic bacteria.

Sr. No	Bacterial Strain	Ampicillin				Methanolic extract			
		Concentration (µg)/ Zone of Inhibition (mm)							
		25	50	75	100	25	50	75	100
1	*Staphylococcus aureus*	20	21	22	23	10	13	16	18
2	*E coli*	17	18	20	21	9	13	16	18
3	*Streptococcus pneumonia*	20	21	22	23	0	0	11	13
4	*Pseudomonas aeruginosa*	17	18	20	21	0	11	13	14

The DPPH assay for anti-oxidant evaluation of D. dissectus methanolic extract

In the method of investigating methanolic extract, it is possible to ascertain the percentage of free radicals that are inhibited at different concentrations in the range of 50, 75, 100, 150, and 250 µg/ml. The DPPH experiment of scavenging free radicals revealed that the methanolic extract of *D. dissectus*, when applied

at different doses, exhibited a higher level of free radical inhibition compared to the positive control ascorbic acid. Based on the curve depicting the IC_{50}, the value of 24.12±0.313 µg/ml is determined for the IC_{50}. An inhibition percentage of 13.79%, 23.06%, 36.29%, 48.58%, and 59.92% was observed in *D. dissectus* methanolic extract at different doses (50, 75, 100, 150, and 250 µg/ml). The IC_{50} value was determined to be 160.03±0.857 accordingly. The concentration of the sample was plotted against the percentage of inhibition,. The methanolic extracts of *D. dissectus* have an IC_{50} value that is more than 36.29 percent, which indicates that they inhibit free radicals in a very powerful way (Table 3).

Table 6. Anti-bacterial activity of the Hexane extract of plant leaves against pathogenic bacteria.

Sr. No	Bacterial Strain	Ampicillin				Hexane extract			
		Concentration (µg)/ Zone of Inhibition (mm)							
		25	50	75	100	25	50	75	100
1	*Staphylococcus aureus*	20	21	22	23	0	9	10	10
2	*E coli*	17	18	20	21	0	0	0	0
3	*Streptococcus pneumonia*	20	21	22	23	0	0	10	12
4	*Pseudomonas aeruginosa*	17	18	20	21	0	10	12	14

The DPPH assay for anti-oxidant evaluation of D. dissectus hexane extract

In the method of investigating methanolic extract, it is possible to ascertain the percentage of free radicals that are inhibited at different concentrations in the range of 50, 75, 100, 150, and 250 µg/ml (Table 4). The DPPH experiment of scavenging free radicals revealed that the hexane extract of *D. dissectus*, when applied at different doses, exhibited a higher level of free radical inhibition compared to the positive control ascorbic acid. Based on the curve depicting the IC_{50}, the value of 24.12±0.313 µg/ml is determined for the IC_{50}. An inhibition percentage of 7.93%, 20.41%, 34.97%, 48.39%, and 56.71% was observed in *D. dissectus* methanolic extracts at different doses (50, 75, 100, 150, and 250 µg/ml). The IC_{50} value was determined to be 145.61±0.628 accordingly. The concentration of the sample was plotted against the percentage of inhibition. The methanolic extracts of *D. dissectus* have an IC_{50} value that is more than 34.97 percent, which indicates that they inhibit free radicals in a very powerful way.

Table 7. Minimum Inhibitory concentration determination for Methanolic plant extract in comparison with Ampicillin.

S. No	Bacteria	Concentration (µg/ml) Ampicillin						Concentration (µg/ml) Methanolic extract					
		5	10	25	50	100	200	5	10	25	50	100	200
1	*Staphylococcus aureus*	0.34	0.30	0.26	0.25	0.19	0.1	0.41	0.39	0.37	0.37	0.29	0.12
2	*E coli*	0.29	0.28	0.28	0.28	0.20	0.11	0.33	0.34	0.32	0.33	0.24	0.20

Total antioxidant capacity (phosphomolybdenum assay)

The total antioxidant capacity (TAC) value was determined using the phosphomolybdenum method. To evaluate absorbance at 695 nm, we used Vitamin C and Gallic acid as benchmarks. The concentration ranges from 25 µg/ml to 400 µg/ml. The standards gallic acid (400 µg /ml -92%), ascorbic acid (400 µg /ml - 90%), methanolic extract of *D. dissectus* (400 µg /ml - 84%), ethyl acetate (400 µg /ml -72%), hexane (400 µg /ml - 68%), and chloroform (400 µg /ml - 60%) exhibited the best Total Antioxidant Capacity (TAC) capacity (Fig. 3) The antioxidant capacity of all solvent fractions exhibited an increase when the concentration of the solvent was increased. The combination consisted of hexane as the remaining component, with a methanol percentage ranging from 60% to 84%.

Determination of anti-bacterial activity

In order to evaluate the effectiveness of *D. dissectus* extracts as antibacterial agents, a number of pathogen

bacterial strains, such as the bacterial strains Gram positive of *Staphylococcus aureus* (ATCC 25923), *Streptococcus pneumonia* (ATCC 33400), and Gram negative of *Pseudomonas aeruginosa* (ATCC 27853), *E. coli* (ATCC 25922) used in the study were obtained from ATCC., were utilized in the study.

Table 8. Anti-fungal activity of various plant extracts with different solvents.

Sr. No	Strain- *Canadia Albicans*	Concentration (µg)/ Zone of Inhibition (mm)			
		25	50	75	100
1	*Fluconazole*	16	17	18	20
2	Methanolic extract	9	10	11	14
3	Extract with hexane	0	9	10	14

Antibacterial activity against Methanolic extract of plant

The antibacterial activity against different pathogenic bacteria was tested with methanolic extracts and found the zone of inhibition shown in Table . The table outlines the findings from an antibacterial activity test, examining the efficacy of methanolic extract against four bacterial strains: *Staphylococcus aureus*, *Escherichia coli* (*E. coli*), *Streptococcus pneumonia*, and *Pseudomonas aeruginosa* using ampicillin as a standard. The concentrations of ampicillin and the methanolic extract are expressed in micrograms (µg), while the corresponding zones of inhibition are measured in millimeters.

In the case of *Staphylococcus aureus,* both ampicillin and the methanolic extract exhibited increasing zones of inhibition with higher concentrations. Ampicillin displayed zones of 20 mm, 21 mm, 22 mm, and 23 mm, while the methanolic extract showed zones of 10 mm, 13 mm, 16 mm, and 18 mm. Similarly, for *E. coli*, both substances demonstrated escalating zones of inhibition with elevated concentrations. Ampicillin displayed zones of 17 mm, 18 mm, 20 mm, and 21 mm, while the methanolic extract exhibited zones of 9 mm, 13 mm, 16 mm, and 18 mm.

Table 9. Percentage of NO Inhibition of Methanol Extract.

Concentration (µg/ml)	% NO Inhibition
Control	100±0
10	4.61±0.112
25	11.27±0.146
50	23.09±0.228
75	38.49±0.362
100	49.04±0.457

Conversely, *Streptococcus pneumonia* responded only to ampicillin, displaying zones of inhibition, while minimal (higher concentration) or no zones were observed for the methanolic extract at lower concentration. For Pseudomonas aeruginosa, ampicillin presented zones of 17 mm, 18 mm, 20 mm, and 21 mm, whereas the methanolic extract demonstrated zones of 0 mm, 11 mm, 13 mm, and 14 mm. The findings suggest varying antibacterial effectiveness of the methanolic extract against different strains, notably active against Staphylococcus aureus and *E. coli*. Comparison with ampicillin provides insights into the relative efficacy of the plant extract in inhibiting bacterial growth.

The absence of zones of inhibition for specific concentrations against certain bacterial strains may indicate limited or no antibacterial effect at those concentrations. Comprehensive investigation and interpretation are required to draw conclusions about the overall antimicrobial potential of the methanolic extract against these bacterial strains.

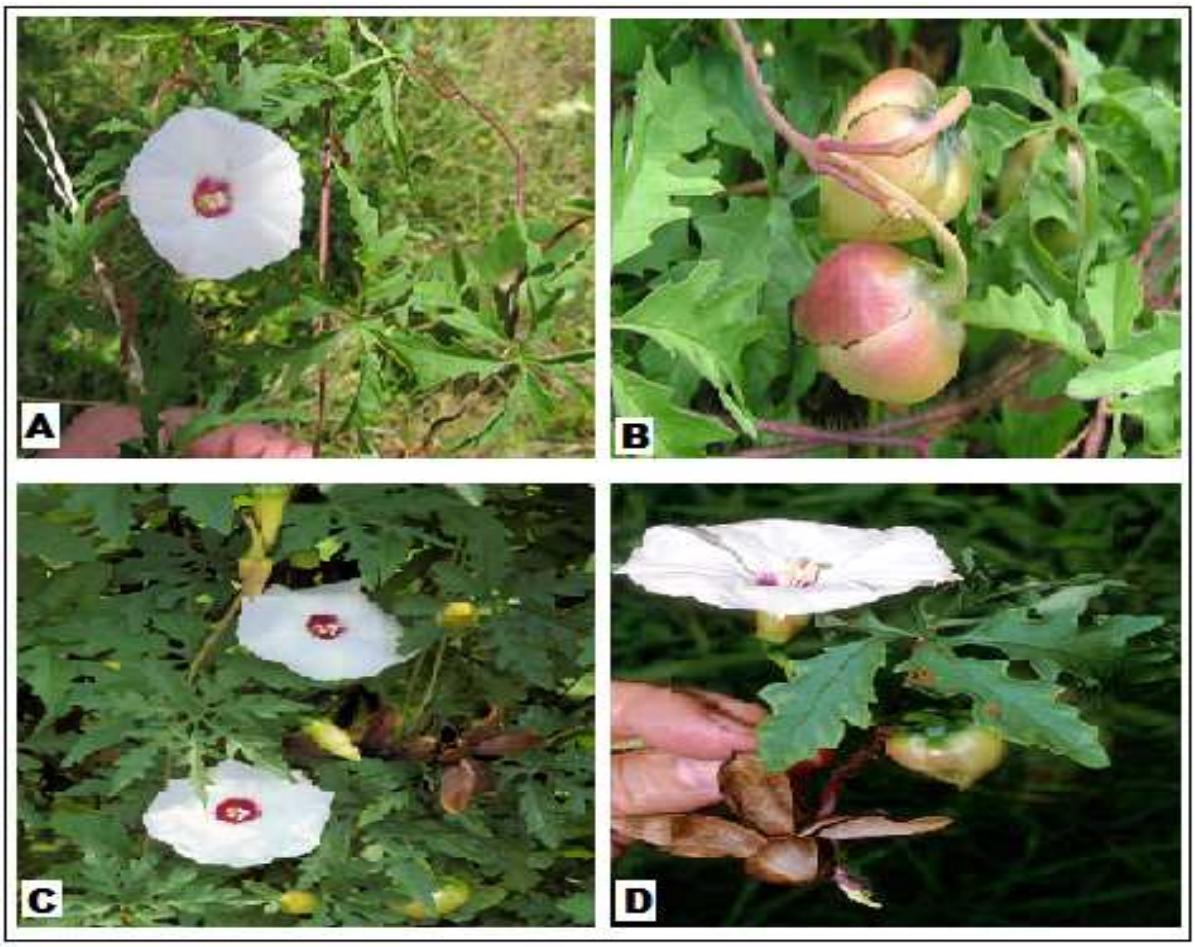

Fig. 1. A-D, Habitat of *D. dissectus.*

Antibacterial activity of plant extract with hexane

The antibacterial activity against different pathogenic bacteria was tested with methanolic extracts and found the zone of inhibition shown in Table . The table presents the outcomes of an antibacterial susceptibility test, evaluating the hexane extract in comparison with ampicillin, a traditional antibiotic. This investigation aimed to assess their effectiveness against four bacterial strains, namely *Staphylococcus aureus, Escherichia coli* (*E. coli*), *Streptococcus pneumonia*, and *Pseudomonas aeruginosa*. The concentrations of ampicillin and the hexane extract are specified in micrograms (µg), with recorded zones of inhibition in millimetres corresponding to each concentration.

For *Staphylococcus aureus*, ampicillin demonstrated a proportional increase in zones of inhibition with higher concentrations, ranging from 20 mm to 23 mm. In contrast, the hexane extract exhibited no inhibitory activity at concentrations 25 µg, and minimal inhibition (9 mm to 10 mm) was observed at higher concentrations. In the case of *E. coli*, ampicillin displayed an ascending trend in zones of inhibition with increasing concentrations, ranging from 17 mm to 21 mm. Conversely, the hexane extract showed no inhibitory effect at any concentration, with all values recorded as 0 mm.

Concerning *Streptococcus pneumonia*, ampicillin revealed an increase in zones of inhibition with rising concentrations, ranging from 20 mm to 23 mm. However, the hexane extract exhibited no inhibition at concentrations below 75 µg, and moderate inhibition (10 mm to 12 mm) was noted at higher concentrations. For *Pseudomonas aeruginosa*, ampicillin showcased an increase in zones of inhibition with higher concentrations, ranging from 17 mm to 21 mm. The hexane extract, on the other hand, displayed no inhibitory effect at concentrations

below 75 µg, with modest inhibition (10 mm to 14 mm) recorded at the highest concentration.

In summary, the results suggest that ampicillin displayed escalating inhibitory effects with increasing concentrations across all bacterial strains. In contrast, the hexane extract exhibited limited or no inhibitory activity, particularly at lower concentrations, against *Staphylococcus aureus*, *E. coli*, and *Streptococcus pneumonia*. *Pseudomonas aeruginosa* exhibited modest inhibition by the hexane extract at higher concentrations.

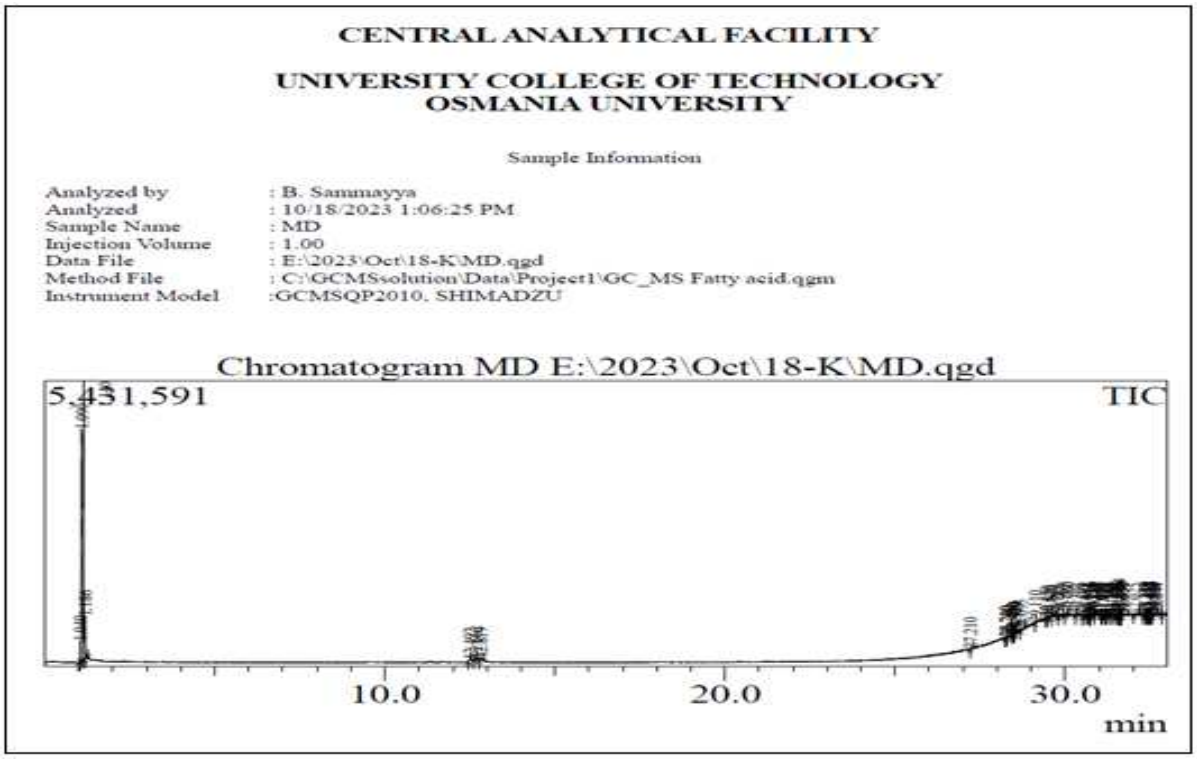

Fig. 2. GC-MS Analysis of Methanolic extract of plant leaves of *D. dissectus*.

Minimum Inhibitory Concentration (MIC) Studies

Minimum inhibitory concentration of methanolic extract of the plant is carried out for bacterial species including *E coli* and *Staphylococcus aureus* using ampicillin as a standard antimicrobial. The study was conducted in nutrient broth using various concentration of test compounds by incubating the samples and standard overnight at 37° C temperature. The results of the MIC studies are presented in **Error! Reference source not found.** and Fig.

The antibacterial activity for *Staphylococcus aureus*, the zone of inhibition (in millimeters) exhibited by ampicillin was 0.34 mm at 5 µg/ml, decreasing to 0.19 mm at 100 µg/ml and further down to 0.1 mm at 200 µg/ml. In contrast, the methanolic extract demonstrated inhibitory effects with zones ranging from 0.41 mm at 5 µg/ml to 0.12 mm at 200 µg/ml.

Similarly, for *Escherichia coli*, the inhibition zones for ampicillin ranged from 0.29 mm at 5 µg/ml to 0.11 mm at 200 µg/ml. The methanolic extract showed inhibition zones ranging from 0.33 mm at 5 µg/ml to 0.20 mm at 200 µg/ml. These results suggest that both ampicillin and the methanolic extract exhibit antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, with varying degrees of effectiveness depending on the concentration used.

Anti-fungal activity

Anti-fungal activity was detected by the dual culture method. *Fussarium oxysporum* NCIM1008 and *Phytophthera infestans* MTCC 8707 were grown on PDA medium. An agar block (five mm diameter) was cut from an actively growing (96 h old) fungal culture and placed on the surface of fresh agar medium at the center of Petri plate.

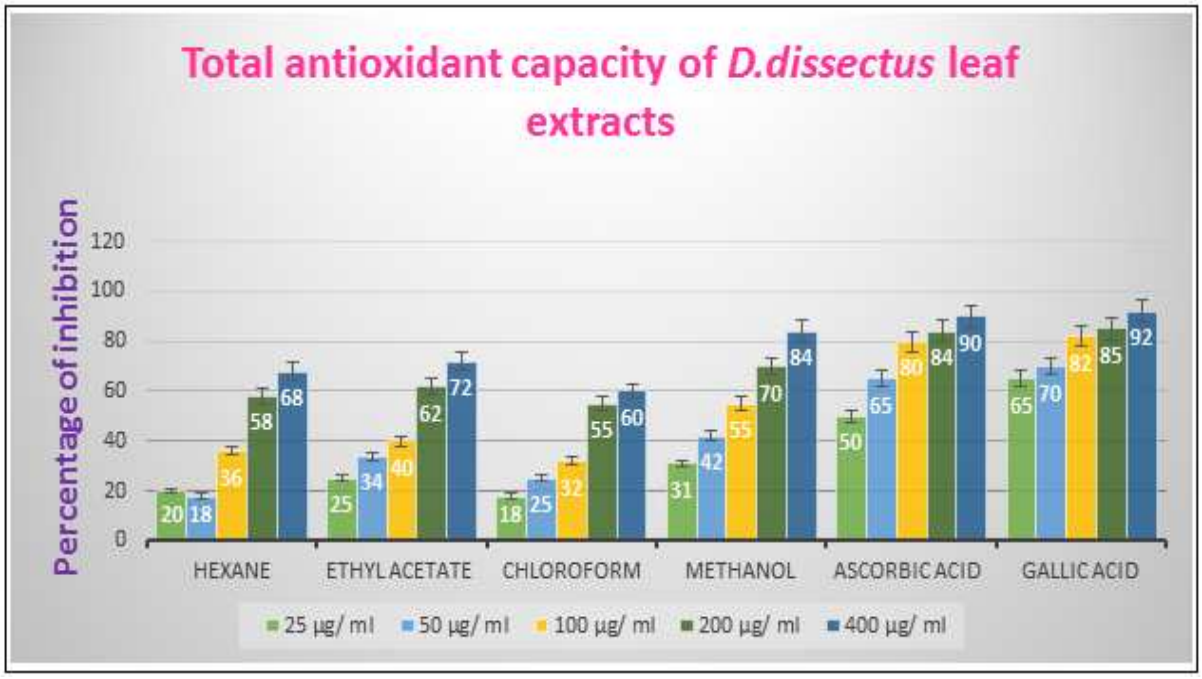

Fig. 3. Graphical representation of image Total antioxidant capacity (phosphomolybdenum assay).

A paper disc dipped in the respective sample was placed onto the plate at different locations of a 90 mm diameter Petri plate and plates were incubated at 30±2 °C. Inhibition zone between two cultures was measured after 5 days of incubation.

The presented table 8 details the results of an antifungal susceptibility test, evaluating the effectiveness of fluconazole, a standard antifungal medication, and two distinct extracts—one derived through methanol and the other through hexane—against the *Candida albicans* strain. The concentrations of fluconazole and the extracts are measured in micrograms (µg), and the corresponding zones of inhibition are documented in millimeters for each concentration.

Fluconazole, the zones of inhibition displayed a gradual increase with higher concentrations, ranging from 16 mm to 20 mm. This suggests a concentration-dependent antifungal impact against *Candida albicans.*

In concern with Methanolic Extract, the methanolic extract exhibited zones of inhibition ranging from 9 mm to 14 mm at concentrations of 25 µg to 100 µg, indicating varying degrees of antifungal activity. The efficacy appears to augment with elevated concentrations. At a concentration of 25 µg, the hexane extract showed no inhibitory activity against *Candida albicans*. However, at concentrations of 50 µg, 75 µg, and 100 µg, zones of inhibition were observed, spanning from 9 mm to 14 mm. This implies a concentration-dependent antifungal effect, with the hexane extract manifesting activity at higher concentrations.

In summary, the findings suggest that fluconazole exhibited a pronounced concentration-dependent antifungal effect against *Candida albicans*. The methanolic extract also displayed antifungal activity, with enhanced effectiveness at higher concentrations. Although initially inactive at the lowest concentration, the hexane extract demonstrated antifungal activity at elevated concentrations, supporting the notion of a concentration-dependent impact against the *Candida albicans* strain.

Anti-inflammatory activity

The anti-inflammatory activity of methanol extract was tested using the Griess reagent by inhibiting the NO production. The percentage of NO inhibition was evaluated at various concentrations of methanolic extract.

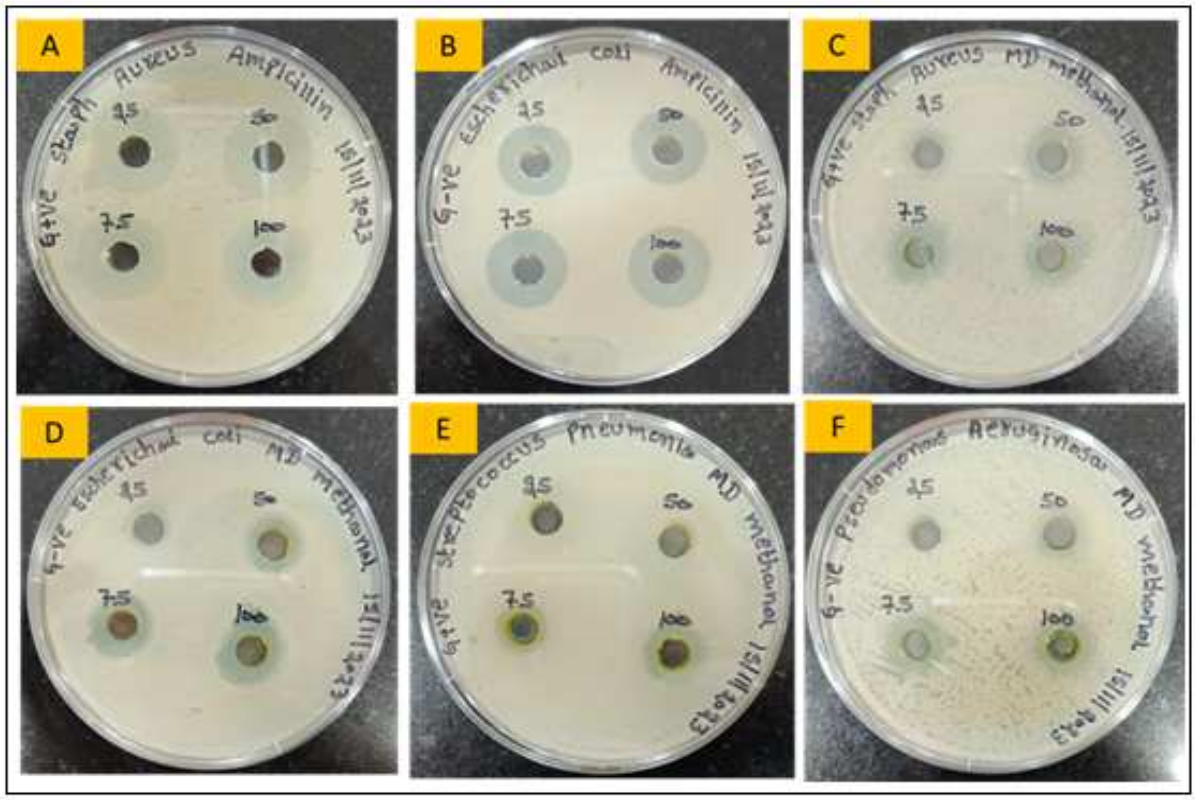

Fig. 4. Anti-bacterial activity against various pathogenic bacteria. (A& B represents zone of inhibition with standard drug ampicillin whereas C, D, E & F are showing zone of inhibition with methanolic extract of plant with pathogenic bacterial stains).

In the control group, there was no inhibition observed, with a 100% baseline. At a concentration of 10 µg/ml, the inhibition of NO was measured at 4.61% ± 0.112. As the concentration increased to 25 µg/ml, the inhibition rose to 11.27% ± 0.146. Subsequently, at concentrations of 50, 75, and 100 µg/ml, the inhibition percentages were recorded at 23.09% ± 0.228, 38.49% ± 0.362, and 49.04% ± 0.457 respectively. The results indicate a dose-dependent relationship between the concentration of the methanolic extract and the inhibition of NO production.

Discussion

An extensive number of plants that are used in herbal remedies are included in the expression "medicinal plants," some of which also possess medicinal properties. These therapeutic plants are regarded as a rich source of materials for the synthesis and development of new drugs. In addition, these plants are essential to the global evolution of human civilizations. Furthermore, certain plants are advised for their medicinal qualities since they are thought to be vital sources of nourishment. Walnuts, ginger, green tea, and a few other plants are among them. Some plants and their derivatives are regarded as significant sources of the active compounds found in threptic purposes (Bassam, 2012). In modern western society, the phrase "alternative medicine" has been widely used and centers on the notion of using plants for therapeutic purposes. The notion that the only medications we can use and trust are those that in pill or capsule form. But the majority of the tablets and capsules we use on a daily basis are made of plant material. Commonly, medicinal plants are used as raw materials to extract the active components needed to synthesize various medications. Certain pharmaceuticals, such as blood thinners, laxatives, antibiotics, and antimalarial drugs, include plant-based components.

Distimake dissectus (Jacq.) A.R. Simões & Staples. is also known as the “alamo vine”, is a perennial herb in the Convolvulaceae family, growing in roadside and

hedge regions. It is distributed to throughout world (Staples *et al.,* 2010). In India like Kerala, Uttar Pradesh, Maharashtra, Andhra Pradesh and Telangana (Sasidharan *et al.,* 2020; Srivastava *et al.,* 2017). The plant was collected in August or September of 2022 from Anantha Giri Hills, which is situated in Telangana State, India's Vikarabad District. This plant has an extensive history of traditional usage in ethnomedicine; the Botanical Survey of India certified their authenticity. Different portions of the plant have been used therapeutically by the indigenous peoples. In order to treat conditions like scabies and the cold, liver disorders, gastrointestinal problems, skin conditions, snakebite, urinary infections, chest problems, and inflammation, Antiarthritic activity traditional healers frequently use the leaves, roots, or extracts from the plant (Rucha Pancham *et al.,* 2020; Austin *et al.,* 2007; Morton 1981; Mansur *et al.,* 2001; Hawthorne *et al.,* 2004; DeFilipps *et al.,* 2004; Ved *et al.,* 2002).

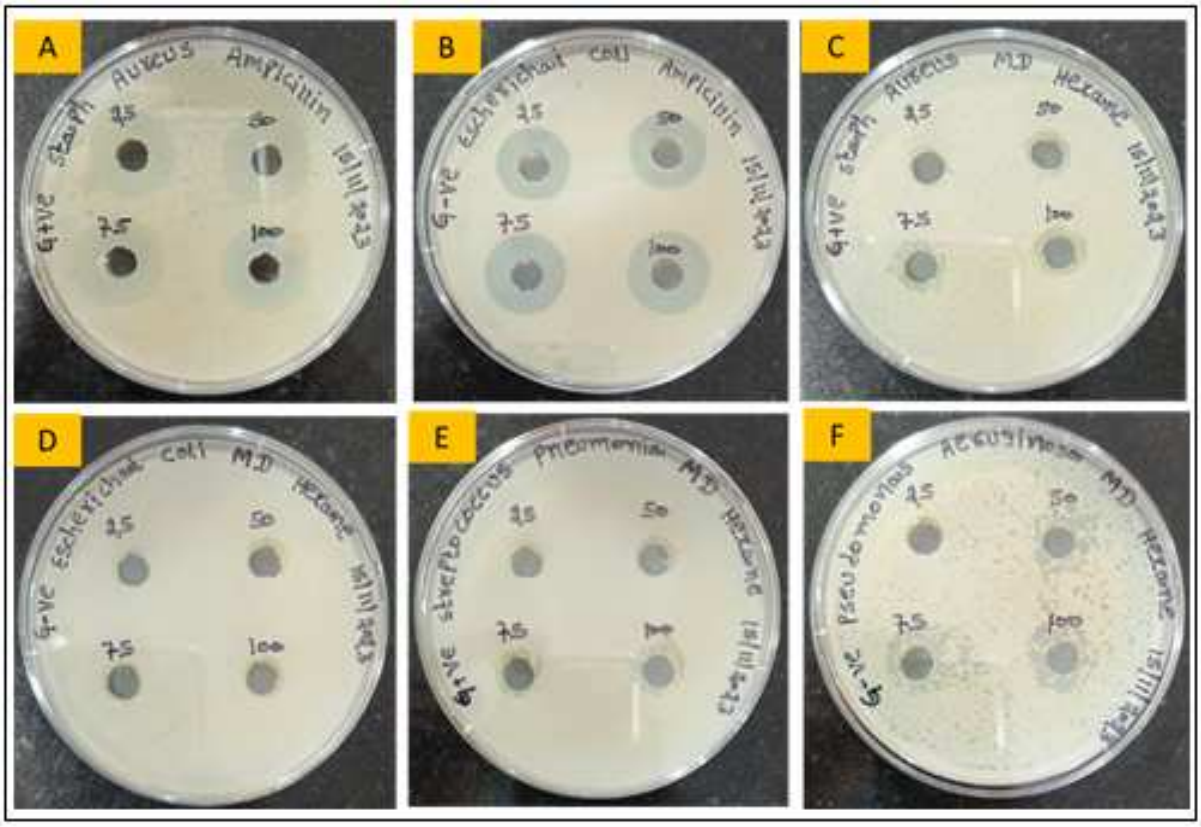

Fig. 5. Anti-bacterial activity against various pathogenic bacteria. (A& B represents zone of inhibition with standard drug ampicillin whereas C, D, E & F are showing zone of inhibition with Hexane extract of plant with pathogenic bacterial stains).

Abdel Karim *et al.,* (2021) reported the GC-MS spectrum of the studied seed oil of *D. dissectus* revealed the presence of 20 components, out of which four is the major constituents, i.e., 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (32.78 %) , Hexadecanoic acid, methyl ester (23.04 %), 9-Octadecenoic acid (Z)-, methyl ester (22.04 %) and Methyl stearate (13.12%). Present studies the methanolic leaf extract of *D. dissectus* was evaluated using GC-MS analysis, which revealed an abundance of 53 phytoconstituents. Among these, 5-major components were 1-chlorofluoroethane ($C_2H_4C_lF$), which has a molecular weight of 82. It accounted for 46.61% of the summit area was made up of it. With a molecular weight of 46, ethanol (C_2H_6O) shown antibacterial action and the second-highest peak area at 23.47%. The peak area of isopropyl alcohol (C_3H_8O), which has a molecular weight of 46, was 5.11%. With a peak area of 0.79%, Germacrene B ($C_{15}H_{24}$) exhibited antifungal action.

Ethoxy-1,1,1,5,5,5-hexamethyl- 3- (trimethylsiloxy) trisiloxane ($C_{11}H_{32}O_4Si_4$), with a molecular weight of 340, has the lowest peak area that was seen, at 0.70%. Abdel Karim *et al.*, (2021) reported Antioxidant activity of D. *dissecta* oil showed 68.08 ± 0.23, and positive control (Propyl Gallate) is 93 ± 0.01. currently the DPPH method's antioxidant activity at different ascorbic acid concentrations, coupled with the outcomes for a control group. Since the control group's samples did not include the test substance or ascorbic acid, there was no antioxidant action, and the percentage inhibition was recorded as 0%. Furthermore, it was shown that ascorbic acid's half-maximal inhibitory concentration (IC_{50}) was 24.12±0.313. The half-maximal inhibitory concentration (IC_{50}) for a methanolic extract was determined to be 160.03±0.857, indicating the concentration at which the extract achieved 50% inhibition of free radicals. Similarly, a hexane leaf extract demonstrated an IC_{50} of 145.61±0.628. These findings are indicative of the antioxidant capabilities of the extracts.

Fig. 6. A: MIC activity of Ampicillin on *S. aureus*, B: MIC activity of Ampicillin on *E. coli*, C: MIC activity of methanolic extract of plant on *S. aureus*, D: MIC activity of methanolic extract of plant on *E. coli.*

The antibacterial activity of *D. dissectus* aqueous extract has been determined. According to Jasim *et al.,* (2018), the extract shown efficacy against both gram-positive bacteria, *Staphylococcus aureus,* and gram-negative bacteria, *Pseudomonas aeruginosa*. *D. dissectus* potential as an antibacterial was examined

in another research. To investigate the leaves' antibacterial properties, petroleum ether, chloroform, benzene, acetone, and ethanol were used in their extraction. According to Theng *et al.,* (2015), the ethanolic extract exhibited the maximum zone of inhibition against all pathogens, including *S. Typhi, P. vulgaris, B. subtilis, E. aerogenes, and P. mirabilis.* The antibacterial activity of Sepals and Fruit was reported by Jawad Ahmad *et al.,* in 2022, while Abdel Karim *et al.,* in (2021) found antibacterial activity on *D. dissectus* seed oils. Present investigation of antimicrobial activity was carried out hexane and methanolic leaf extract against pathogenic bacteria's *S. aureus, E. coli, S. pneumonia and P. aeruginosa and* ampicillin as a standard. Ampicillin and methanolic extract suppressed *Staphylococcus aureus* at higher dosages. The methanolic extract exhibited 10mm, 13mm, 16mm, and 18mm zones, whereas ampicillin had 20mm, 21mm, 22mm, and 23mm zones. Both methanol and ampicillin increased *E. coli* inhibitory zones with concentration. The methanolic extract contained 9 mm, 13 mm, 16 mm, and 18 mm zones, whereas ampicillin had 17 mm, 18 mm, 20 mm, and 21 mm zones. *S. pneumonia* only inhibited by ampicillin, but the methanolic extract at lower doses revealed no zones. For *P. aeruginosa*, ampicillin revealed zones of 17 mm, 18 mm, 20 mm, and 21 mm, whereas the methanolic extract showed 0 mm, 11 mm, 13 mm, and 14 mm. *S aureus* and *E. coli* appear to respond differently to the methanolic extract. Comparing the plant extract to ampicillin indicates its bacterial inhibition. In contrast, the hexane extract showed no inhibitory action at 25 µg and modest inhibition (9-10 mm) at higher dosages. *E. coli* ampicillin inhibition zones increased from 17mm to 21 mm with increasing doses. The hexane extract had no inhibitory impact at any concentration, with all values at 0 mm.

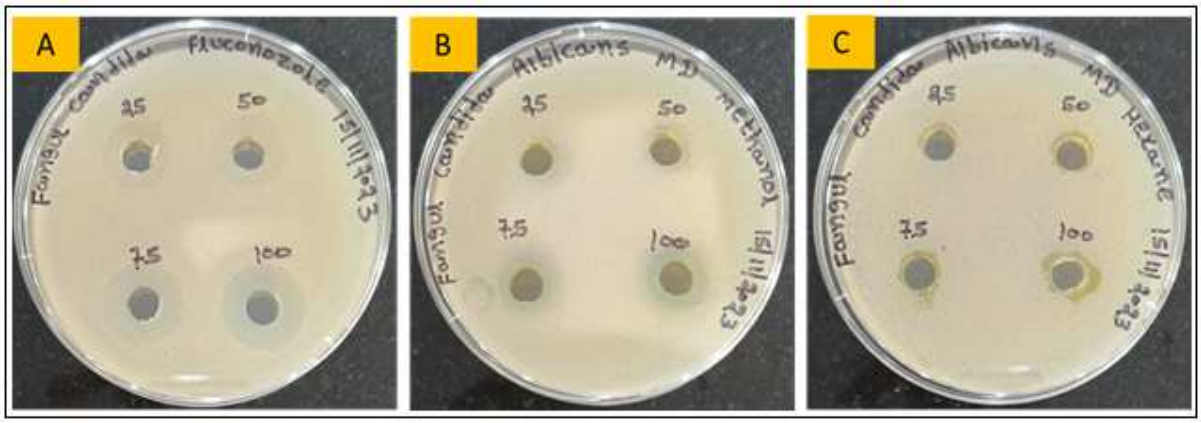

Fig. 7. Anti-fungal activity, A represents for fluconazole against *candida albicans* whereas B and C represents for methanolic extract and hexane extract of plant against *candida albicans* respectively.

Increasing ampicillin concentrations increased zones of inhibition in Streptococcus pneumonia from 20 to 23 mm. The hexane extract showed no inhibition below 75 µg, while substantial inhibition (10mm -12 mm) was seen at higher dosages. High ampicillin doses increased zones of inhibition for *P. aeruginosa* from 17mm to 21 mm. The hexane extract showed no inhibitory effect below 75 µg, but showed mild inhibition (10-14 mm) at the maximum dose. The minimum inhibitory concentration (MIC) for *S. aureus* and *E. coli* by ampicillin varied from 0.34 mm at 5 µg/ml to 0.19 mm at 100 µg/ml and 0.1 mm at 200 µg/ml, respectively, with methanolic extract showing similar results.

Joshi *et al., (*2015) reported evaluated for antifungal activity the plant was extracted with different solvents and tested against fungi viz *Candida albicans* and *Aspergillus niger*. Alcoholic extract showed maximum zone of inhibition as compared to other extracts. Current antifungal activities hexane and methanol activities against fungi viz *Candida albicans,* and fluconazole used as standard. The findings suggest that fluconazole exhibited a

pronounced concentration-dependent antifungal effect against *Candida albicans*. The methanolic extract also displayed antifungal activity, with enhanced effectiveness at higher concentrations. Although initially inactive at the lowest concentration, the hexane extract demonstrated antifungal activity at elevated concentrations, supporting the notion of a concentration-dependent impact against the *Candida albicans* strain.

The anti-inflammatory efficacy of methanol extract was assessed using the Griess reagent, which inhibited NO generation. NO inhibition was measured at different methanolic extract concentrations. Baseline inhibition was 100% in controls. The inhibition of NO at 10 µg/ml was 4.61% ± 0.112. 25 µg/ml increased inhibition to 11.27% ± 0.146. Inhibition percentages at levels of 50, 75, and 100 µg/ml were 23.09% ± 0.228, 38.49% ± 0.362, and 49.04% ± 0.457, Concentration-dependent NO suppression by methanolic extract.

Conclusions

In summation, the findings of this study unravel the intricate pharmacological profile of *D. dissectus* and underscore its potential as a reservoir of herbal remedies for a myriad of health conditions. The nuanced understanding of its bioactive constituents and their pharmacological actions opens new vistas for drug discovery and the development of innovative therapeutic interventions rooted in natural medicine. Through continued exploration and translational research, *D. dissectus* may emerge as a cornerstone in the quest for novel pharmacotherapeutic agents, contributing to the advancement of holistic healthcare paradigms worldwide.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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