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Synthesis, Characterization, Antimicrobial, Antioxidant and Anticancer Activity of Silver Nanoparticles of Euphorbia Thymifolia

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ABSTRACT:
The present study reveals that E.thymifolia plant used for medicine or tonic properties.E.thymifolia belongs to the family Euphorbiaceae, which has around 7500 species in about 300 genera. The plants under Euphorbia genus are used to treat cancer, migraine, warts, intestinal parasites, tumors, etc. The use of E.thymifolia in curing many ailments are increasing as more and more properties of this plant is being found due to advanced research. The E.thymifolia is found in tropical regions, but it is absent in North Australia.
In India, the plant is found in the hills and plains. E.thymifolia is found usually two ecotypes as green and red forms. The traditional use of this E.thymifolia is mainly due to its actions involving laxative, aromatic, sedative, blood purification, anti-viral, antihelminthic, anti-inflammatory, anti-spasmodic, anti-fungal, anti-bacterial, antimicrobial, diuretic properties etc. E.thymifolia is a small branched, hispidly pubescent, prostate annual herb, commonly known as laghududhika or choti-dudhi. The leaves, seeds and fresh juice of whole plant are used in worm infections, as stimulant, astringent. It is also used in bowel complaints and in many more diseases. Recent investigations have proven that secondary metabolites from natural resources containing bioactive components have a wide variety of biological properties. This study provides significant evidence about the biological activity of different extracts of E.thymifolia. These results indicate that E.thymifolia extract can be good source for antioxidant, antimicrobial and anticancer. Therefore, further studies to isolate and identify of bioactive compounds from E.thymifolia extract for in vitro investigations of observed activities are highly recommended. In addition, it is necessary to elucidate the mechanisms of action of these extracts and bioactive compounds isolated from this plant at the cellular and molecular level to evaluate biological capacity of substances on specific therapeutic properties.
Keywords: *E.thymifolia, Euphorbiaceae, antihelminthic, laghududhika, anti- inflammatory, anti-spasmodic, anti-fungal, anti-bacterial, anti-microbial.*

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1. INTRODUCTION:

EUPHORBIA THYMIFOLIA
AND ANTICANCER ACTIVITY OF SILVER NANOPARTICLES OF
SYNTHESIS, CHARACTERIZATION, ANTIMICROBIAL, ANTIOXIDANT
E.thymifolea Leaf (Euphorbiaceae) is a small branched, hispidly pubescent, prostate
microbial, diuretic properties etc antihelminthic, anti-inflammatory, anti-spasmodic, anti-fungal, anti-
bacterial, anti-to its actions involving laxative, aromatic, sedative, blood purification, anti-viral,
calcifuges and facultative calcicoles. The traditional use of this E.thymifolia is mainly due
intermediates. These are categorized into two major ecological groups like obligate
These two forms interbreed among themselves and results in forming three

Fig.-1 E.thymifolia

E.thymifolia is found usually two ecotypes as green and red forms.
conditions, abandoned fields, etc. In India, the plant is found in the hills and plains.
Australia. This plant is present in the wastelands, along roadsides and wall sides in humid
advanced research. The E.thymifolia is found in tropical regions, but it is absent in North
ailments are increasing as more and more properties of this plant is being found due to
migraine, warts, intestinal parasites, tumors, etc. The use of E.thymifolia in curing many
species in about 300 genera. The plants under Euphorbia genus are used to treat cancer,
or choti-dudhi. E.thymifolia belongs to the family Euphorbiaceae, which has around 7500

permanent blindness; whilst ingestion can cause purging or more severe problems.
causing irritation and blistering; contact with the eyes causing temporary or even
for this plant, the latex in most, if not all Euphorbias is caustic and toxic-skin contact often
also sold in local markets. Known Hazards Although we have seen no specific information
and also in many other areas of the tropics. Itis commonly harvested from the wild and is
numerous adventitious roots. The plant is a very popular medicinal herb in much of Africa
a prostrate annual plant producing stems up to 25 cm long. The stems usually produce
used in bowel complaints and in many more diseases. General Information E.thymifolia is
fresh juice of whole plant are used in worm infections, as stimulant, astringent. It is also
annual herb, commonly known as laghududhika or choti-dudhi. The leaves, seeds and

against Herpes simplex virus type 2 in vitro in a dose-dependent manner.
shown to possess antioxidant activities. Some have also shown significant activity
 Several extracts from the whole plant as well as several pure compounds have been
scabei mite, which causes scabies.
 The ether extract of the whole plant showed significant activity against the Sarcoptes
verrucosum in vitro and in vivo in calves.
activity against the dermatophytes Trichophyton mentagrophytes and Trichophyton
 The ethanol and water extracts of the whole plant showed significant antifungal
found to be active against S.flexneri in vivo.
growth of Escherichia coli and S.flexneri in vitro. The ethyl acetate extract was also
 Ethyl acetate and chloroform extracts of the aerial parts of the plant inhibited the
 Gram positive (B.subtilis) and Gram-negative (E.coli) bacteria.
furthermore have antiviral, anti-allergic, anti-inflammatory, and antitumour properties.
haemostatic, antithrombic, antioxidant and vasoprotective actions. The flavonoids
 Both flavonoids and tannins have been reported to have anti-inflammatory,analgesic,
flavonoids.
dried whole plant a range of hydrolysable tannins have been isolated as wellas several
 The plant is rich in medically active compounds. From an aqueous acetone extractof the
enteritis, diarrhoea and venereal diseases.
 The plant is widely used in Africa in decoction or infusion as a treatment for dysentery,

1.1 APPLICATION OF EUPHORBIA THYMIFOLIA

eczema and skin inflammations.
 A decoction of fresh aerial parts is applied externally to treat dermatitis, measles,
hypertension, absence of menstruation and venereal diseases.

 A decoction of the whole plant is drunk to treat lung problems, fever, influenza,
 The ground fresh leaves are rubbed in to treat intercostal pain.
 The leaves are pulped with water and applied to the head to treat headache
stimulate contractions of the uterus.
 A maceration of the dried leaves is drunk for facilitating childbirth; it is claimed to
 The leaves are used in a decoction to treat cystitis and kidney ailments
 An infusion of the leafy stems is taken as a bitter diuretic
and venereal diseases.
 Plant and seed decoctions are also taken as a galactagogue, and to treat hypertension
antihelmintic and laxative.
 The dried leaves and seeds are slightly aromatic and are used as a stimulant, astringent,
off flies and mosquitoes, and a vermifuge for dogs.
 The essential oil is put into medicinal soaps for treatment of erysipelas, sprays tokeep
spraying the plants first with water extracts of whole E.thymifolia plants.
 In pot tests, infection of vigna radiata by tomato spotted wilt virus was preventedby
against a range of human tumour cell lines.
 Several derivatives of the tannin galloyl-β-D-glucose show significant cytotoxicity

 It is used in the treatment of eye swellings, discharge and conjunctivitis.
scabies.
 The latex is applied externally to treat to warts and as a remedy for ringworm and
 Women with heavy menstruation drink the latex as a tonic.
rubefacient products to treat alopecia.
enable them to carry loads on their heads as adults, and also as mild irritating
 Crushed plants are rubbed on the scalp for strengthening the skull bones of children to
are applied as a plaster for healing sprains.
 The decoction is also used as an eye wash to treat conjunctivitis. Fresh crushed plants

 To perform the anti-cancer activity(Liver cancer cell line-HepG-2).
 To check the anti-oxidant activity.
 To detect anti-bacterial activity.
 To isolate the bacterial culture from suitable source.
SEM
 To synthesis of Silver Nanoparticle and Characterization by using UV, FTIR,
 To analyse Phytochemicals in the leaf extracts.
 To extract the leaf using suitable solvents.
 To collect the E.thymifolia leaf from the local area of Coimbatore.

II. AIM AND OBJECTIVES

CHAPTER 2
c. 8 mm long; glands 4. Male flowers 1-4, ebracteolate.Female laterally pendulous; ovary pale green and often pinkish in color. Cyathia in axillary clusters. Involucre campanulate, apex and smooth toward the base and venation is reticulate. Petiole is small, thin, slender, oval-oblong or obliquely oblong. Apex is obtuse or rounded. Margin is dentate towards coppery red when fresh, becoming grayish green or dark purplishon drying. The lamina is unequal sided at base. The petiolate, 3-6 mm long, 2-4 mm wide, mostly green, but often oblong or ovate, 4-8 mm long and 2-5 mm wide with rounded apex, oblique base, small, branches radiating, slender, reddish and pubescent. Leaves are simple,opposite, elliptic, white latex, spreading on the ground, 10-20 cm in length with a diameterfrom 1 to3 mm; color when fresh, becoming grayish green or dark purplish on drying. Stems are with hispid prostrate herbs. Stem puberulous, slender, cylindrical, pale green but often pink in for this plant, the latex in most, if not allEuphorbias is caustic and toxic-skin contact Softly also sold in local markets. Known Hazards although we have seen no specific information and also in many other areas of the tropics. It is commonly harvested from the wild and is numerous adventitious roots. The plant is a very popular medicinal herb in much of Africa a prostrate annual plant producing stems up to 25 cm long. The stems usually produce used in bowel complaints and in many more diseases. General information E.thymifolia is fresh juice of whole plant are used in worm infections, as stimulant, astringent. It is also annual herb, commonly known as laghududhika or choti-dudhi. The leaves, seeds and

E.thymifolea Leaf (Euphorbiaceae) is a small branched, hispidly pubescent, prostate
3.1 REVIEW OF LITERATURE
CHAPTER 3

late 1970s used silver particles for the treatment of orthopedic diseases caused by
their attractive and unique physiochemical properties. Researches carried out in the
respiration. AgNPs are widely used in biology and medicine especially because of
revealed that AgNPs have the ability to attach to cell walls and alter cellular
on living cells and, so, are unable to cause microbial resistance. Recent studies
important part of nanotechnology mainly because they do not induce modification
exposed to different types of microbes increases considerable . AgNPs are a very
antimicrobial effectbut when silver nanoparticles (AgNPs) are used the surface area
majority of cases, silver participates in its nitrate form, thus inducing a strong
microorganisms and, therefore, have multiple roles in the medical field .In the
being used in numerous antimicrobial applications. Silver ions are highly toxic for
years, as a metal that exhibits good medical properties, silver-based compounds
developing field of nanotechonology. Silver has been known, for more than 2000
and morphology and, therefore, are a very important component in the rapidly
Nanoparticles have unique properties as a consequence of their size, distribution
properties and many more.
inflammatory, anti-spasmodic, anti- fungal, anti-bacterial, anti-microbial, diuretic
laxative, aromatic, sedative, blood purification, anti-viral, anti-helminthic, anti-
E.thymifolia is one of the many medicinal plants which have various actions like
among all the plants found all over the world many plants are found in India.
treated. Many plants and trees are found to have various medicinal values and
effective in their mode of action and do not cause any side effects to the patient
traditional method in many parts of the world. The plant extracts are found to be
The use of plant extracts to cure many diseased conditions has been the
reddish brown without caruncle.
are conical, log, ovoid and obtusely quadrangular, up to 1 mm long, acutely 4-angled,
sessile capsule 1 mm × 1 mm base truncate, short-hairy.They are cocci when mature. Seeds
tomentose; style 3-forked from base. Fruits are ovoid- globose, acutely 3-lobed, almost

whole grains, etc.
concentrated in colourful parts of the plantslike fruits, vegetables, nuts, legumes, and
phytochemicals discovered and in most cases, these phytochemicals are
epicatechins, and proanthocyanidins. In total, there has been over 25,000
and isoflavones, and flavanols. Flavanols further are classified as catechins,
based on their similarchemical structure, such as anthocyanins, flavones, flavanones,
flavonoids, and stilbenes/lignans. Flavonoids can be further divided into groups
categories, such as carotenoids and polyphenols, which include phenolic acids,
established yet. Phytochemicals under research can be classified into major
essential nutrients because proof of their possible health effects has not been
Phytochemicals generally are regarded as research compounds rather than
reagent.
does not require high pressure, energy, temperature or the use of toxic chemical
multiple advantages overclassical routes: it is cost effective, eco – friendly and
yeasts) or different alcoholic or aqueous plant extracts. Green synthesis has
methods use eitherbiological microorganisms (e.g.: bacteria, fungi, marine algae,
have become more attractive ways to obtain AgNPs. These unconventional
wasteful purifications.In recent years green chemistry and biosynthetic methods
hazardous chemicals, low compound conversions, high energy requirements and
so no chemical, radiation and microwave-assisted routes) they usually involve
micelles, thermal decomposition of different silver compounds, electrochemical,
obtain AgNPs (e.g.: solution, chemical / photochemical reactions in reverse
solar energy, food additives, textile industry etc., conventional methods used to
chemical reactions, bio-labeling, spectral selective coatings for the absorption of
Many other applications can be attributed to AgNPs, for example: catalysts in
different infections with microorganisms and a fasterbone recovery was noticed.

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certain types of porous media have been developed to kill microbeson contact.

resulted in solutions that can go beyond simply inhibiting microbial growth. Instead, bacterial growth. In response, further advancements in antimicrobial technologies have bactericidal agents, which kill bacteria, and bacteriostatic agents, which slow down or stall broadened to include all antimicrobials. Antibacterialagents can be further subdivided into as a synonym for them by medical professionals and in medical literature), its context has fluoroquinolones. Though the term used to be restricted to antibacterials (and is often used microorganisms but is now also applied to synthetic agents, such as sulfonamides or "antibiotic" originally described only those formulations derived from living during surgery), and antibiotics (which destroy microorganisms within the body). The term spread of illness, antiseptics (which are applied to living tissue and help reduce infection as bleach), which kill a wide range of microbes on non-living surfaces to prevent the The main classes of antimicrobial agents are disinfectants (non-selective agents, such is known as antimicrobial prophylaxis.

antimicrobial chemotherapy, while the use of antimicrobial medicines to prevent infection bacteriostatic agents. The use of antimicrobial medicines to treat infection is known as microbes are microbicides, while those that merely inhibit their growth are called used against fungi. They can also be classified according to their function. Agents that kill primarily against. For example, antibiotics are used against bacteria, and antifungals are Antimicrobial medicines can be grouped according to the microorganisms they act An antimicrobial is an agent that kills microorganisms or stops their growth.

any given biological activity.

often complex, and identifying what specific phytochemical is primarily responsible for that field include isolating specific compounds and determining their structures,which are cultures, in vitro experiments, or in vivo studies using laboratory animals. Challenges in followed by defining their structure or testing in laboratory model systems, such as cell Phytochemicals by first extracting and isolating compounds from the origin plant,

3.1.Phytochemisty study

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they include: fundamental vibrations based on crystals and molecules; fixed-point and the aromatic compounds. The LRM and FTIR methods have the same characteristics; spectroscopy is mainly used in the analysis of conjugated diolefine, carbonyl compounds, The absorption spectrum of UV has higher sensitivity and lower detection limit. UV disease.

supplementation with selenium or vitamin E does not reduce the risk of cardiovascular E have no positive effect on mortality rate or cancer risk.needs update Additionally, be effective at preventing disease. Supplements of beta-carotene, vitamin A, and vitamin Antioxidant dietary supplements have not been shown to improve health in humans, or to and cosmetics, and being oxidation-inhibitors in rubber, synthetic plastics, and fuels. The former, industrial antioxidants, have diverse uses: acting as preservatives in food and naturally occurring compounds that are present in foods and tissue.

groups of substances: industrial chemicals that are added to products to prevent oxidation, vitamin C and vitamin E. The term "antioxidant" is mostly used for two entirely different catalase and superoxide dismutase), produced internally, or the dietary antioxidants complex systems of overlapping antioxidants, such as glutathione and enzymes (e.g., these chain reactions. To balance the oxidative stress, plants and animals maintain cells of organisms. Antioxidants such as thiols or ascorbic acid (vitamin C) terminate that can produce free radicals, thereby leading to chain reactions that may damage the Antioxidants are compounds that inhibit oxidation. Oxidation is a chemical reaction prolonged antibiotic treatment, as for recurrent Clostridioides difficile infections.

Stool transplants may be considered for patients who arehaving difficulty recovering from Consumption of probiotics and reasonable eating may help to replace destroyed gut flora. decrease the number of enteric bacteria, which may have a negative impact on health. pharmacodynamics, and chemicalcomposition. Prolonged use of certain antibacterials can classification within these categories depends on their antimicrobial spectra, as beta-lactams, macrolides, quinolones, tetracyclines or aminoglycosides. Their Antibacterials are used to treat bacterial infections. Antibiotics are classified generally

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ingestion
can
cause
purging
or
more
severe
problems.
blistering; contact with the eyes causing temporary or even permanent blindness; whilst information about the surface topography and composition. Often causing irritation and the beam interact with the sample, producing various signals that can be used to obtain (SEM) scans a focused electron beam over a surface to create an image. The electrons in chemical properties of the substances in the inclusions. A scanning electron microscope microregion analysis of solid, gas, and liquid inclusions; quantitative analysis of the

c.Test for reducing sugars (Fehling's Test)
solution and observed for the formation of deep blue / black colour.
About 2ml of the extract was treated with 10% ferric chloride
b.Test for Phenol (Ferric chloride test)
the presence of Alkaloids
Wagner's reagent. The formation of reddish brown precipitate confirms
About few ml of plant extract was treated with 4-5 drops of
a.Test for Alkaloids (Wagner's test)
by using below standard tests.
The qualitative phytochemical study was performed on the extracts
4.3 PHYTOCHEMICAL ANALYSIS
was then stored and used.
and then subjected to extraction using Soxhlet apparatus. The extract
The dried leaves were coarsely powdered using an electric mixer
4.2 SOLVENT EXTRACTION
for about 10-15 days.
and washed in water to remove dust. Then the leaves were shade dried
The plant leaves were collected from the outskirts of Coimbatore
4.1 PLANT COLLECTION
4.MATERIALS AND METHODS
CHAPTER 4

g.Test for Aminoacids and Proteins (Ninhydrin test)
bluish green colour indicates the presence of phytosterols
amount of concentrated sulphuric acid was added. The formation of
few drops of acetic anhydride were added. To that mixture added equal
1ml of the plant extract was treated with 2 ml of chloroform and
f.Test for Phytosterols (Salkowski's Test)
presence of flavonoids.
solution was added. The formation of green or blue colour indicates the
To about 2ml of plant extract, few drops of 10% ferric chloride
e.Test for Flavonoids
presence of saponins.
persistent foam for few seconds. The presence of foam confirms the
mixture was shaken vigorously and observed for the formation of
To 2 ml of the plant extract added 6ml of water in a test tube. The
d.Test for Saponins (Foam test)
for the appearance ofblue colour.
mixture was boiled in a boiling water bath for 10 minutes and observed
To 1 ml of the extract added few drops of Fehling's reagent and the

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plant extract was measured by using 2, 2-diphenyl-1-picrylhydrazyl The free radical scavenging activity of methanolic extract of
4.4.1. DPPH radical scavenging activity
4.4ANTIOXIDANT ACTIVITY
acid. Formation of red colour indicates the presence of glycoside
To 1ml of plant extract added few ml of concentrated sulphuric
j.Test for glycosides
of dark green or blue color.
chloride solution. The presence of tannin is confirmed by the formation
To about 1ml of plant extract added few drops of dilute ferric
i.Test for Tannin
precipitate indicates the presence of steroids.
was added to 1ml of flower extract. The formation of red colour
About 2ml of chloroform and 0.2ml of concentrated sulphuric acid
h.Test for Steroids
amino acids and proteins
reagent. A purple or violet colour formed indicates the presence of
To a few ml of plant extract added small amount of Ninhydrin

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solution in 40mmol/l HCl plus 2.5ml of 20mmol/l FeCl3.6H2O and
water bath. The FRAP reagent contained 2.5ml of 20mmol/l TPTZ
samples and reagent blank were incubated at 37ºC for 30mins in a
and 30µl of test sample, or acetone (for the reagent blank). The test
freshly and incubated at 37ºC, was mixed with 90µl of distilled water
as modified by Pulido et al. (2000). FRAP reagent (900µl), prepared
according to the procedure described by Benzie and Strain (1996) and
The antioxidant capacity of plant extracts samples were estimated
4.4.2. Ferric reducing/antioxidant power (FRAP) assay
A518 (control)
activity % = _____=0.45
Scavenging
A518 (control) - A518 (sample) x100
the experimental tubes.
was determined by comparing the absorbance values of the control and
The percentage inhibition of DPPH radicals by the extract/compound
0.1ml of respective vehicle in the place of plant extract/ascorbic acid.
measuring the absorbance at 517 nm. A control was prepared using
temperature for 30 min. Decolorization of DPPH was determined by
was shaken vigorously and allowed to reach a steady state at room
extract/ascorbic acid at various concentrations were mixed. The mixture
of 3ml of 0.004% DPPH solution in methanol and 0.5to2.5µl of plant
according to the procedure described by (Braca et al., 2001). An aliquot
(DPPH) The scavenging activity for DPPH free radicals was measured

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control
sample
control
% Inhibition= (A
-A
)/A
)×100
by the following formula:
performed in triplicates, and their percentage inhibition was calculated
finally absorbance is observed at 540nm. All the reactions were
w/v)). This was kept on incubation at room temperature for 30mins, and

sulfanilic acid and 200µL naphthylethylenediamine dichloride (0.1%) 0.5mL of above solution, 0.5mL of Griess reagent was added (0.1mL of This reaction mixture was kept for incubation at 25°C for 150mins. To of PBS (pH-7.4) and 100µL of different concentration of plant extract. determined by adding 400 µL of 100mM sodium nitroprusside, 100µL NO scavenging activity (Garrat DC., 1964) of sample was

4.4.3. Nitric oxide (NO) scavenging activity

activity

A518 (control)

Scavenging= _____=91%

A518 (control) - A518 (sample)x0422

(II) solution determined using the corresponding regression equation. to the theoretical absorbance value of a 1 mmol/l concentration of Fe antioxidant giving an absorbance increase in the FRAP assay equivalent 1mmol/l FeSO4.7H2O. EC1 was calculated as the concentration of antioxidant has a ferric- TPTZ reducing ability equivalent to that of Equivalent Concentration (EC1) was defined as the concentration of used for the preparation of the calibration curve. The parameter concentration ranging between 100 and 2000µmol/l (FeSO4.7H2O) was immediately at 593nm using a spectrophotometer. The known Fe (II) the end of incubation period the absorbance readings were recorded 25ml of 0.3mol/l acetate buffer, pH 3.6 (Benzie and Strain, 1996). At

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15mins.

assay. The media was adjusted to the pH and autoclaved at 121°C for (LB) were used throughout the study for determining the antibacterial Muller-Hinton Agar (MHA), Nutrient Broth (NB) and Luria Britani

4.5.1Media and culture condition

species are bacterial cultures.

pseudomonas species, bacillus species, klebsiella species; proteus species were selected viz., Escherichia coli, staphylococcus aureus, To perform antimicrobial activity using various bacterial and fungal

4.5ANTIBACTERIAL ACTIVITY

% Inhibition = (A control-A sample) /A control) X 100

carried out in triplicates. Percent inhibition of the assay was calculated. at 230nm. BHT was taken as a positive control and the reaction was pH-7.4). This was incubated for 10 minutes, and absorbance was read 4mM hydrogen peroxide solution prepared in phosphate buffer (0.1M, al. 1989. 850µL of the aqueous plant extract was added to 150µL of This activity of the plant was evaluated by the method of Ruch et

2

4.4.4. Hydrogen peroxide scavenging activity (H

O

)

2

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and control plates were also maintained. Zone of inhibition was were incubated at 37°C for 24 hours. Assay was carried into triplicates 50 µl of plant extract was poured into the wells. After that, the plates were dipped into required solvents then placed in agar plates. Then 10- bacterial inoculums was swabbed uniformly. The sterile paper discs sterile petriplates. After solidification of media, 20-25µl suspension of MHA plates were prepared by pouring 20ml of molten media into extracts was determined by Well Diffusion method (Bauer et al., 1996). The antibacterial activity and antifungal activity of crude extract

4.5.3Well Diffusion method

separately at 37°C for 18-20hrs for bacterial culture.

organism was transferred into 50ml nutrient broth and incubated cultures were kept at 4°C. For use in experiments, a loop of each test potato dextrose slant were incubated at 27°C for 3-5 days. These stock nutrient agar media slants were incubated at 37°C for 24 hours and incubated at 27°C for 3-5 days. A single colony was transferred to agar. Bacterial cultures then incubated at 37°C for 24 hours and fungal subsequently stroke out on nutrient agar media and potato dextrose incubated at 27°C for 3-5 days. Each suspension of test organism was 24hours and at 37°C on shaking incubator and fungal cultures were nutrient broth bacterial cultures were incubated with agitation for transferring a loop full of cells from stock cultures to test tubes of 50ml potato dextrose agar. Active culture for experiments were prepared by Stock cultures were maintained at 4°C on slopes of nutrient agar and

4.5.2 Inoculum Preparation of Bacterial Culture

21

samples. They were initially dissolved in neat dimethylsulfoxide After 24hrs the cells were treated with serial concentrations of the test

2

attachment at 37

C, 5% CO

, 95% air and 100% relative humidity.

0

at plating density of 10,000 cells/well and incubated to allow for cell microlitres per well of cell suspension were seeded into 96-well plates containing 5% FBS to give final density of 1x10

cells/ml. One hundred

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cells were counted using a hemocytometer and diluted with medium tetra acetic acid (EDTA) to make single cell suspensions and viable The monolayer cells were detached with trypsin-ethylene diamine

Cell treatment procedure

medium was changed twice a week.

humidity. Maintenance cultures were passaged weekly, and the culture were maintained at 37

C, 5% CO2, 95% air and 100% relative

0

Essential Medium containing 10% fetal bovine serum (FBS). The cells Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum The human Liver cancer (Hep-G2) was obtained from National

Cell line

4.6 In-Vitro Anticancer Activity

measured from the clear zone in mm.

about 24hrs and control was also maintained. Zone of inhibition was extract were poured on to wells. Then plates were incubated at 37°C for agar. Well were put into the agar medium using sterile forceps. Plant suspension was spread on muller-hinton agar plate and potato dextrose measured from the edge of the well to the zone in mm. The tested cell

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as follows
The percentage cell viability was then calculated with respect to control
measured the absorbance at 570nm using micro plate reader.
formazan crystals were solubilized in 100µl of DMSO and then
4hrs. The medium with MTT was then flicked off and the formed
buffered saline (PBS) was added to each well and incubated at 37
C for
0
After 48hrs of incubation, 15µl of MTT (5mg/ml) in phosphate
the number of viable cells.
Therefore, the amount of formazan produced is directly proportional to
tetrazolium ring, converting the MTT to an insoluble purple formazan.
enzyme in living cells, succinate-dehydrogenase, cleaves the
(MTT) is a yellow water soluble tetrazolium salt. A mitochondrial
3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium
bromide
MTT assay
triplicate was maintained for all concentrations.
medium containing without samples were served as control and
2
48hrs at 37
C, 5% CO
, 95% air and 100% relative humidity. The
0
Following sample addition, the plates were incubated for an additional
of medium, resulting in the required final sample concentrations.
dilutions were added to the appropriate wells already containing 100 µl
sample concentrations. Aliquots of 100 µl of these different sample
Additional four serial dilutions were made to provide a total of five
desired final maximum test concentration with serum free medium.
(DMSO) and an aliquot of the sample solution was diluted to twice the

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software.
Log concentration and IC50 was determined using Graph pad prism
Nonlinear regression graph was plotted between % Cell inhibition and
Cell Inhibition = 100- Abs (sample)/Abs (control) x100.
The % cell inhibition was determined using the following formula. %
% Cell viability = [A] Test / [A]control x 100

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Flavanoids
Green (+++)
brown (+++)
Alkaloids
Reddish
PHYTOCHEMICAL TEST
RESULTS
Table:1 -TABULATION OF PHYTOCHEMICAL ACTIVITY
indicates the presence of glycoside.
by the formation of dark green or blue color. Formation of red colour
indicates the presence of steroids. The presence of tannin is confirmed
of amino acids and proteins. The formation of red colour precipitate
of phytosterols. A purple or violet colour formed indicates the presence
flavonoids.The formation of bluish green colour indicates the presence
formation of green or blue colour indicates the presence of

seconds. The presence of foam confirms the presence of saponins. The appearance of blue colour. formation of persistent foam for few blue / black colour. Presence of reducing sugars observed for the precipitate confirms the presence of Alkaloids for the formation of deep extracts by using below standard tests. The formation of reddish brown The qualitative phytochemical study was performed on the

5.1 PHYTOCHEMICAL ANALYSIS

RESULTS

CHAPTER 5

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and Silver nitrate synthesised E.thymifolia

Fig.2 difference between E.thymifolia leaf Extract

Saponins

Present foam(++)

Phenol

Deep blue colour(+++)

Reducing sugar

Blue colour(+++)

Tanin

Blue colour(+++)

Phytosteroids

Bluish green (+++)

Ancinoacids&proteins

Negative(-)

Precipitate(++)

Steroids

Red

colour

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