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A REVIEW ON ANTIMICROBIAL ACTIVITY OF MEDICINAL PLANTS

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ABSTRACT: The herbal medicine have been used as healing properties. Microbial resistance to classical antibiotics and it is rapid progression have raised series concern in the treatment of infection disease. It shows the anti-microbial activity plant of common name, botanical name, medicinal application, biological compounds. It is reasonable to investigate the microbial survival and the antimicrobial potential via a variety of method. In the phytochemical review major group of phytochemicals are classified as Alkaloids, Phenolic and Polyphenol, Quinones, Flavones ,flavonoids and flavonols, Tannins. Screening methods have Agar diffusion method, Disk diffusion method, Agar cup method and Turbidimetric method. It also include Mechanism of antimicrobial activity. This information is intended to help the researchers and field experts to select suitable method for testing the hygienic safety and anti-microbial properties.

Keywords: Anti-microbial activity, Screening, Phytochemical, Methods, Mechanism.

I. INTRODUCTION:

In ancient times the herbal medicine have been used as healing properties. These plants contain medicinal value with bio-active compounds, such as alkaloids, tannins, flavonoids and phenolic compounds [1]. The medicinal properties of different plant which results in unique specific properties. The essential oil of any herb can be extracted using a different methods including distillation, solvent extraction, cold pressing, maceration (or) supercritical carbon extraction[2].The maximum benefits of oils are demonstrate with pharmacological uses[3].

The anti-microbial study was conducted to determine the properties in some medicinal plants. Eg: Guava *(psidium guajava*), Green tea *(camellia sinensis)*, Neem *(azadira indica),* Marigold *(calendula officinalis*) and Thulsi *(Ocimum sanctum).* The primary objective of the study was to aid to the progressive research works related to the anti-microbial activity of plants[4].

LIST OF SOME ANTI-MICROBIAL ACTIVITY PLANTS ARE MENTIONED BELOW:

S.no	Common name	Botanical name	Medicinal applications	Biological compounds	Reference
1	Clove	*Syzygium aromaticum*	Antioxidant, antimicrobial, anti-inflammatory , anti-mutagenic, anti-allergic and anti-cancer.	Eugenol, eugenyl acetate, α-humulene, 2-heptanone, and βcaryophyllene	[5,6]
2	Portulaca	*Portulaca oleracea*	Antioxidant, antimicrobial, anti-inflammatory, anticancer, neuroprotective and	Ascorbic acid, a-tocopherols, omega-3 fatty acids, apigenin, gallotannins,	[7,8]

			antidiabetic.	quercetin, and kaempferol	
3	Tribulus	*Tribulus terrestris*	Antioxidant, antimicrobial, analgesic, anti-inflammatory and cardiovascular protective.	flavonoid, tannin and phenolic acids	[9,10]
4	Eryngium	*Eryngium maritimum*	Antioxidant, antimicrobial, anticancer, antidiabetic, antimalarial, anti-Alzheimer and anti-inflammatory.	Flavonoids, phenolic acids, and coumarins	[11]
5	Cinnamon	*Cinnamomum verum*	Antioxidant, antimicrobial, anti-inflammatory, anticancer, cholesterol-lowering, immunomodulatory and cardiovascular.	Cinnamaldehyde and eugenol	[12-14]
6	Turmeric	*Curcuma longa*	Antioxidant, antimicrobial, anti-inflammatory, anticancer, hypoglycemia and anticoagulant.	Vitamin-C, cineole, tumerone, borneol, zingiberene, d-sabinene, and d-phellandrene	[15,16]
7	Ginger	*Zingiber*	Antioxidant, antimicrobial, anti-diabetic, neuro protective, analgesic, cardiovascular, gastrointestinal, anti-inflammatory, anticancer and antihypertensive.	Phenolic acids, gingerols, paradols and Shogaols	[17,18]
8	Thyme	*Thymus vulgaris*	Antioxidant, antimicrobial, expectorant, spasmolytic, mucolytic and antitussive.	Carvacrol, thymol and phenols	[19,20]
9	Pennyroyal	*Mentha pulegium*	Antioxidant, antimicrobial, anti-hepatic, Anti-genotoxic.	Neo-menthol, pulegone and menthone	[21,22]
10	Fennel	*Foeniculum vulgare*	Antioxidant, antimicrobial and anti-inflammatory	Phenolic compounds	[23]
11	Chamomile	*Matricaria chamomilla*	Antioxidant, antimicrobial, anti-inflammatory, anticancer, analgesic, anti-hypoglycemic, anti-stress and hepatoprotective.	Flavonoids, terpenoids, phenolic compounds, apigenin and matricin	[24,25]
12	Mint	*Mentha*	Antioxidant, antimicrobial, anticancer and	Phenolic compounds	[26]

			anti-inflammatory.		
13	Eucalyptus	*Eucalyptus globulus*	Antioxidant, antimicrobial anti-inflammatory and antipyretic.	Flavonols, hydroxybenzoic acids and hydrolyzable tannins	[27,28]
14	Primrose	*Primula vulgaris*	Antioxidant, antimicrobial, anti-neuropathic, anti-inflammatory, anticancer and anti-ulcerogenic.	Phenolic acids, flavonoids, sterols, hydrocarbons, and tocopherols	[29,30]
15	Lemon balm	*Melissa officinalis*	Antioxidant, antimicrobial, anti-stress, anti-Alzheimer, anti-inflammatory, anticancer, anti-cardiovascular and antispasmodic.	Phenolic componds such as thymol and Carvacrol	[31,32]

PHYTOCHEMICAL REVIEW :

Over thousand of years, the phytochemicals are have the large numbers of chemical compounds that are naturally occurring in plants, confering colour, flavours, aroma and texture, that are defend organisms from the affect of virus, bacteria, fungi, free radicals.

Some of major groups of phytochemicals are classified based on their chemical structures are listed below :

1) Alkaloids.
2) Phenolics and polyphenols.
3) Quinones.
4) Flavones, flavonoids, and flavonols.
5) Tannins**.**

ALKALOIDS:

Alkaloids area unit an enormous and structurally various cluster of secondary metabolites that have microorganism, plant, or animal origins. they'll be found in around three hundred plant families. However, some compounds area unit restricted to specific families, like poison in asterid dicot family (Cushnie et al., 2014). although they're gift in several components of the plant, bound compounds area unit restricted to a {particular} part, like antimalarial drug in cinchona bark. Alkaloids are found in terrestrial and in some marine animals. There area unit over eighteen,000 alkaloids from completely different sources (Dembitsky, 2005). Alkaloids area unit heterocyclic structures containing one or additional atomic number 7 atoms. they're classified supported their chemical structure or natural origin. Since some alkaloids area unit restricted to bound sources, classification because of their natural origin is possible (Cushnie et al., 2014). There area unit 2 broad divisions within the classification consistent with the chemical structure. the primary division contains the non-heterocyclic or atypical alkaloids, additionally known as protoalkaloids or biological amines, like hordenine or N-methyltyramine, colchicine, and Ethril (an antibiotic; Evans, 2009). The second division includes the heterocyclic or typical alkaloids like hygrines happiness to the pyrrole and pyrrolidine cluster, and antimalarial drug happiness to the quinoline cluster. The second division will be split into fourteen teams supported the ring structure (Evans, 2009). the most structural units of alkaloids area unit shown in Figure a pair of. There area unit many ways that to call individual alkaloids. they'll be named consistent with the organism from that they're isolated; for instance, antidote springs from belladonna (Cushnie et al., 2014). once many alkaloids area unit discovered from constant supply, this needs the usage of a prefix or a additional complicated suffix, for instance, quinine, hydroquinine, quinidine, or a series of letters, for example, epicoccarine A,

and epicoccarine B. Finally, alkaloids will be named supported their pharmacologic activity (for example, emetine induces vomiting), the name of the supply geographic location (for example, tasmanine was isolated from a Australian state plant),or when the name of their discover (for example, pelletierine when academic. Pelletier; cushnie et al.,2014).Alkaloids area unit proverbial in each ancient and fashionable medication to possess many pharmacologic activities. Some alkaloids hane been integreated into human culture as recreational medication or abusive medication like alkaloid, cocaine, and vasoconstrictive. Some alkaloids area unit proverbial to be extremely poisonous, resulting in many cases of human poisoning (cushnie et al.,2014).Alkaloids will kind element bonds with catalyst, receptors, and proteins, since they need, additionally to practical teams, a nucleon acceptive atomic number 7 atom and one or perhaps additional nucleon donating paraffin element atoms. Alkaloids have several pharmacologic properties, like system stimulant (brucine), anticholinergic agents (atropine), medicament and agent activity (ergometrine), and antimalarial drug activity (quinine;cushine et al.,2014).Table a pair of Summarizes a number of the most alkaloids found in geographic area Plants with bactericide activity.

PHENOLICS AND POLYPHENOLS:

The bioavailability of polyphenols, or the quantity of polyphenols that's absorbed unchanged, typically determines its biological activity. Polyphenols might additionally have the systema digestorium while not being absorbed, so touching internal organ microbiota. this will result in 2 consequences: 1st, polyphenols area unit changed into their active form; second, they alter the composition of the internal organ microbiota, most likely inhibiting unhealthful microorganism and enriching helpful microorganism. Thus, polyphenols have a major impact on the human host health (Abbas et al., 2017). they'll be divided into many teams. The structures of main phenoplast compounds and their derivatives area unit shown in Figure one. straightforward Phenols and phenoplast Acids straightforward phenols and phenoplast acids vary from being a straightforward phenol ring with one substitution like cinnamic and caffeic acids to having multiple substitutions and hydroxylations. there's proof that the positioning and degree of hydroxylation correlates with the toxicity of the secondary substance. The substance looks to be additional repressive the additional oxidised the structure is (Cowan, 1999). Mechanisms of inhibition of phenolics embrace inhibiting enzymes. it's urged that this inhibition takes place through reactions with sulfhydryl teams on the proteins (Cowan, 1999; Coppo and Marchese, 2014). Four plants from Lebanese flora, cloves (Eugenia caryophyllata), mint (Mentha piperita), rosemary (R. officinalis), and cherry (Prunus avium) extracts were tested for his or her antimicrobial activity exploitation the broth microdilution technique against B. subtilis. Cloves had the best bactericide activity (MIC of one.6 and 0.6 mg/ml when liquid extractions at eighty and 100 ◦ C, respectively), followed by cherry (MIC of four and a pair of.4 mg/ml when extractions at eighty and 100 ◦ C, respectively). Phytochemical analysis incontestible the presence of phenolics in cloves, cherry, and rosemary. there have been additionally indication of flavonoids and terpenoids in cloves and rosemary (Shehadi et al., 2014). Ethanolic extracts of 3 Saudi Arabian plants were tested for antimicrobial activity. The fruits of asterid dicot genus incanum L., leaves of castor-oil plant L., and wild leek power unit. porrum L. were tested against 9 strains of microorganism. All 3 plants incontestible bactericide activity against S. aureus, E. coli, P. aeruginosa, Acinetobacter sp., K. pneumoniae, Proteus sp., Micrococcus sp., cocci epidermidis, and B. subtilis. The ethanolic leaf extract of R. communis exhibited the best bactericide activity with inhibition zones go between seventeen.46 and 27.22 metric linear unit at a degree of twenty mg/ml and MIC of ten mg/ml (Alamri and Moustafa, 2012). The ethanolic leaf extract of A. ampeloprasum power unit. porrum followed with a spread of inhibition zones between thirteen.33 and twenty three metric linear unit at a degree of twenty three mg/ml and MIC of eleven.5 mg/ml. the best activity was against P. aeruginosa and Micrococcus sp. Phytochemical screening exploitation HPLC confirmed the presence of 5 phenoplast compounds as well as phenoplast acids cinnamic acid, p-coumaric acid, and ferulic acid additionally as catechin and sinapic acid (Alamri and Moustafa, 2012). Ethanolic extracts of Syrian propolis were able to inhibit growth of all S. aureus strains tested as well as MRSA strains. They were additionally able to inhibit A. baumanii with AN inhibition zone of around fifteen metric linear unit at 2 hundredth concentration. The ethanolic extract was less economical on P. aeruginosa and E. coli strains. Propolis extracts contain many active compounds as well as phenoplast acids and phenoplast aldehydes additionally as flavonoids and quinones (Harfouch et al., 2016). Matricaria aurea L., native to Saudi Arabia, may be a herb additionally referred to as golden Anthemis nobilis. Antimicrobial screenings exploitation many techniques as well as agar well-diffusion assay, tube dilution assay, and scanning microscopy discovered larger inhibition zones in methanolic extracts than in ethanolic ones. B. subtilis was the foremost sensitive to the methanolic extracts with AN inhibition zone of twenty four.83 mm, followed by S. pyogenes (23 mm), S. aureus (21 mm), and K. pneumoniae (21 mm; Rizwana et al., 2016). Methicillin-resistant S. aureus (MRSA) strains, E. coli, and E. faecalis incontestible moderate sensitivity compared to the opposite strains

tested. Antifungal activity was additionally assayed; Colletotrichum gleosporoides was the foremost sensitive with inhibition zones between fifty and sixty six.22 metric linear unit for various extracts. MICs with methanolic extract were zero.4 mg/ml for B. subtilis, K. pneumoniae, and S. aureus and the next MIC of fifty mg/ml for MRSA strains. MICs for plant strains were between zero.2 and 6.35 mg/ml for Alternaria alternata and C. gleosporoides and the next MIC of twelve.5–50 mg/ml for A. niger and genus Aspergillus flavus. Scanning microscope (SEM) imaging showed that the extract-treated cells incontestible a modification in form and size additionally as agglomeration. Cell harm and destruction was determined when treatment for twelve and twenty four h. GC-MS analysis confirmed the presence of phenols and phenoplast acids (Rizwana et al., 2016). Pomegranate (P. granatum L). growing across the center East was tested for many healthful uses as well as antimicrobial activity. Pomegranate juice is made in polyphenols, particularly caffeic acid, acid, and epigallocatechin gallate (the main active element in inexperienced tea). Hydrochloric extracts of pomegranate slashed the colony-forming units/ml of plaque microorganisms by 84%; these microorganisms embrace Aggregatibacter actinomycetemcomitans, P. intermedia, and P. gingivalis (Bhandari, 2012).

QUINONES:

Quinones area unit characterised by having AN aromatic ring with 2 organic compound substitutions. compound antimicrobial activity comes from their ability to present free radicals. they'll additionally kind irreversible complexes with amino acids in proteins, so inactivating them. These properties of quinones build it attainable for them to attack surface adhesions, polypeptides within the cell membrane, and membrane enzymes. Quinones may sequester substrates needed by the microorganisms (Cowan, 1999). Well-diffusion assay analysis of Lawsonia inermis Linn usually referred to as henna incontestible bactericide activity against P. aeruginosa, with the best activity shown by species from the Al-Sharqiya region (Sultanate of Oman). Henna leaves contain up to five of 2-hydroxy-1,4-naphthaloquinone by weight. The presence of compound offers it its coloring properties (Habbal et al., 2011; Rathi et al., 2017). many quinones gift in Roman coriander seeds from everywhere the center East tested to possess antimicrobial result against S. aureus, S. mutans, and strep mitis. Methanolic fractions had inhibition zones go between fifteen and thirty metric linear unit, whereas n-hexane fractions ranged between zero and twenty two metric linear unit with variations between countries of origin. This antimicrobial activity is attributed to compounds like thymoquinone, thymohydroquinones, and dithymoquinone (Sudhir et al., 2016). liquid and methanolic extracts of the Lebanese custard apple L. seeds area unit made in quinones additionally as phenols and flavonoids. The extracts demonstrate AN MIC of fifty mg/ml against S. aureus, E. faecalis, S. epidermidis, E. coli, and P. aeruginosa. They additionally demonstrate AN MBC of a hundred mg/ml against the antecedently mentioned strains (Nasser et al., 2017)

FLAVONES, FLAVONOIDS, AND FLAVONOLS:

Flavones include associate aromatic ring with just one organic compound substitution. Hydroxylation yields a flavonol. Flavonoids occur as a C3–C6 unit joined to a phenolic resin ring that's additionally hydroxylated. These compounds area unit legendary to be synthesized in plants as a process against microorganisms; therefore, their antimicrobial result is of no surprise. Their antimicrobial properties area unit in all probability as a result of they kind complexes with each animate thing and soluble proteins, similarly as microorganism cell membrane. they may additionally disrupt cell membranes if oleophilic enough (Cowan, 1999). Phytochemical screening of the methylene chloride and ester fractions of Premna resinosa full-grown in Asian country|Asian country|Asian nation} showed the presence of flavonoids. Agar diffusion assay incontestible sturdy antimicrobial activity, with the best activity within the methylene chloride fraction. MICs for the methylene chloride fraction ranged between zero.01 and one mg/mL against S. aureus, B. subtilis, E. coli, S. typhimurium, E. faecalis, enterobacteria flexneri, and A. baumanii. gram-positive strains were additional vulnerable than gram-negative strains (Albadawi et al., 2017). The antimicrobial activity of Phoenix dactylifera L. (Date palm) growing in Al Madina, Saudi Arabia, was evaluated by analysis through MIC followed by scanning microscopy against imipenem-resistant P. aeruginosa (IRP). Active compounds were determined to be flavonoid glycosides, together with quercetin, apigenin, and luteolin. The MIC of the choloroform fraction was zero.05 mg/ml which of the MBC was a pair of mg/ml. Biofilms made by twelve IRP isolates were utterly eradicated with five-hitter extract for one h. Analysis with SEM, when applying the flavonoid glycosides, incontestible that P. aeruginosa cells began to deform at thirty min. At 60 min, the cells were utterly malformed, therefore suggesting that the mechanism of action is thru forming pores within the cell membrane and damaging it (Selim et al., 2012). Thyme leaves (Thymus vulgaris) and myrrh exudates (Boswellia carterii), employed in ancient drugs in Asian country, were tested against seven microorganism species together with S. aureus, B. cereus, and

bacteria pneumophila similarly as 2 plant life species (A. flavus and Fusarium oxysporum). MICs ranged between2 and four-dimensional (v/v). Phytochemical screening incontestible the presence of rosmarinic, caffeic, chlorgenic acid, carnosol, and flavonoids (Al-Juraifani, 2009). Saadabi et al. (2006) tested seventy eight plant extracts from twenty six plants growing in Asian country for antimicrobial activity. Alkaloids, flavonoids, and tannins were gift in 5 of the foremost active plants together with Plicosepalus acaciae, gourd vine, family Cyperaceae rotrdus, Nymphea lotus, and Vahila dichotoma. The chloroform extracts of P. acaceiae (against P. aeruginosa), M. balsamina (against P. aeruginosa), C. rotrudus (against E. coli), N. lotus (against B. subtilis), and V. dichotoma (against S. aureus) had inhibition zones of eighteen, 15, 15, 16, and 14 mm, severally (Saadabi et al., 2006). Olive (O. europaea) leaves and rocket (Eruca sativa) seeds native to Asian country and Palestine were analyzed against S. aureus and B. cereus. Screening confirmed the presence of flavonoids within the extracts. Methanolic extracts incontestible inhibition zone diameters of 3–8 millimeter. MICs for olive and rocket extracts were eighty and 60μg/ml, severally, against S. aureus, and forty and 20μg/ml against B. caryophylloid dicot genus (Malik, 2015). Activities of neem (neem), common ginger (ginger), fever tree, R. officinalis, and L. inermis were analyzed for antimicrobial activity against gram-positive microorganism (B. subtilis, S. aureus, and M. roseus) and gram-negative microorganism (E. coli, K. pneumoniae, enterobacteria dysenteriae, and P. aeruginosa). Inhibition zones ranged between eight and twenty nine millimeter against Gramnegative microorganism with the strongest activity exhibited by A. indica against E. coli. Inhibition zones against gram-positive microorganism ranged between five and eighteen mm; the strongest activity was exhibited by R. officinalis and L. inermis against M. roseus and by E. globulus against S. aureus. MICs ranged between fifty and 200μg/ml with the strongest activity by A. indica against E. coli, P. aeruginosa, and S. dysenteriae. Phytochemical screening of the assorted plant extracts indicates the presence of flavonoids and tannins similarly as saponins, steroids, and anthocyanins (El Sayed and Aly, 2014). Yemeni lawsoniainermis L. leaves were extracted exploitation many solvents together with methyl alcohol, ethanol, acetone, and water. Phytochemical screening showed that each one the leaves contained flavonoids similarly as quinones, tannins, and alkaloids. resolvent extracts incontestible the best medication activity with inhibition zones of thirty six, 17, 30, 34, and twenty four millimeter against B. subtilis, E. coli, L. monocytogenes, Kocuria rhizophila, and S. epidermidis, severally, at a level of 250 mg/ml. Methanolic extract incontestible slightly weaker medication activity followed by ethanolic extract. The binary compound extracts incontestible little or no or no medication activity (Al Maqtari and Al. Maqtari, 2014).

TANNINS:

Tannins area unit a bunch of chemical compound phenolics. they're divided into 2 main categories: chemical reaction and condensed tannins. acid forms the idea of chemical reaction tannins, sometimes esterified at multiple locations with D-glucose. Condensed tannins area unit additional bumper and area unit derived from flavonoid monomers; they will be cited as proanthocyanidins (Cowan, 1999). Tannins' biological activity can be correlative to the patterns of chemical reaction and chemical process (Coppo and Marchese, 2014). Tannins exert their antimicrobial result by complexing with proteins through each valency and non-covalent interactions. they're additionally capable of complexing with polysaccharides. proof additionally exists for direct inactivation of microorganisms; it absolutely was shown that low concentrations of tannins modified the morphology of the germ tubes of Crinipellis perniciosa. within the case of condensed tannins, they need additionally been shown to be capable of binding cell walls of ruminal microorganism, inhibiting growth, and peptidase activity (Cowan, 1999). Olea sp. growing within the Albaha region in Asian country incontestible antimicrobial activity in each its binary compound and ethanolic extracts. Ethanolic extracts exhibited higher associatetimicrobial activity with an repressive zone of twenty five millimeter against gram-positive microorganism. MICs against S. aureus, E. coli, S. pyogenes, enterobacteria sp., and P. aeruginosa ranged between thirty one.2 and 62.5 μg/ml. Phytochemical screening of Olea sp. incontestible a high concentration of tannins similarly as flavonoids, steroids, terpenoids, and coumarins (Khayat et al., 2018). Conocarpus erectus L., a tropical and semitropical evergreen tree cultivated in Asian country, was evaluated exploitation crude extracts from varied components of the plants similarly as pure tannins. Tannins were active against 3 plant life species: yeast, A. niger, and Penicillium chrysogenum with inhibition zones of fourteen.3, 12.5, and 13.3 mm, severally (Shohayeb et al., 2013). Alcoholic extracts of the flowers, fruit, leaf, and stem of the plant incontestible activity solely against S. cerevisiae with inhibition zones of eleven.3, 13.3, 10.3, and 11.0 mm, severally. once tested against microorganism, flowers and fruits of C. erectus were additional active than alternative components of the plant. Generally, Grampositive microorganism together with S. aureus

and B. subtilis incontestible higher sensitivity than gram-negative microorganism. gram-positive inhibition zones ranged between twenty one and twenty three millimeter and MICs ranged between zero.21 and 1.33 mg/ml. Inhibition zones of gram-negative microorganism ranged between eleven and eighteen millimeter and MICs between zero.42 and eight mg/ml. imperviable microorganism eubacteria phlei had inhibition zones between sixteen and seventeen millimeter and MICs between zero.33 and 2.33 mg/ml (Shohayeb et al., 2013). Methanolic and binary compound extracts of Z. officinale and herbaceous plant growing in Asian country exhibited antimicrobial activity. Z. officinale reserved S. pyogenes, S. aureus, E. coli, and P. aeruginosa growth with inhibition zones of ten, 10, 9, and 14 mm, severally, for the binary compound extract and twelve, 12, 10, and 12 mm, severally, for the methanolic extract. C. longa displayed inhibition zones of eleven, 11, 11, and fourteen millimeter for the binary compound extract and nineteen, 15, 12, and twelve millimeter for the methanolic extracts, severally. Phytochemical screening indicated the presence of tannins within the extracts of the 2 plants (Al-Daihan et al., 2013). The methanolic extracts of pomegranate (P. granatum) peel extracts contain high concentrations of chemical reaction tannins, ellagic acid, and acid. The extracts exhibited activity against E. coli O157:H7, enterobacteria spp., true bacteria cholerae and L. monocytogenes (Coppo and Marchese, 2014).

S.NO	METHOD	STRENGTH	LIMITATION
1	**Agar dilution**	1. Low cost 2. Doesn't require specialized lab facility 3. Use equipment and reagent readily available in microbiology lab 4. Can be perform by most lab staff	1. Hydrophobic extracts may separate out from agar . 2. Inoculum size, presence of solubilizing agent and incubation temperature can affect zone of inhibition. 3. Volatile compounds can affect bacterial and fungal growth in closed environments. 4. Data is only collected at one or two time points. 5. Some fungi are very slow growing.
2	**Disk well diffusion**	1. Low cost Results available in within 1–2 days. 2. Does not require specialized laboratory facilities. 3. Uses equipment and reagents readily available in a microbiology laboratory. 4. Can be performed by most laboratory staff. Data is only collected at one or two time points. 5. Large numbers of samples can be screened. 6. Results are quantifiable and can be compared statistically.	1. Differential diffusion of extract components due to partitioning in the aqueous media. 2. Inoculums size, presence of solubilizing agents, and incubation temperature can affect zone of inhibition. 3. Volatile compounds can affect bacterial and fungal growth in closed environments.
3	**Broth dilution**	1.Allows monitoring of activity over the duration. 2.More accurate representation of antibacterial activity. Micro-broth methods can be used to screen large numbers of samples in a cost-effective manner.	1. Essential oils may not remain in solution for the duration of the assay, emulsifier and solvent may interfere with accuracy of results. 2. Labor and time intensive if serial dilution are used to determine cell count 3. Highly colored extracts can interfere with colorimetric endpoints in micro broth methods.

4	**TLC bioautograpy**	Simultaneously fractionation and determination of bioactivity	1. Unsuitable where activity is due to component synergy 2. Dependent on the extraction methods and TLC solvent used.

ENDPOINT SCREENING METHODS

AGAR DIFFUSION:

The agar diffusion test has been probably most widely used one of the endpoint test. It is otherwise known as disk assay. This diffusion has many variations such as cylinder,well, the ditch plate, agar overlay.Among the foremost common variations of the assay, the antimicrobial compound is applied to an agar plate, mistreatment AN inseminated paper disk, or placed in a very well. The compound diffuses through the agar, fixing a level gradient. The concentration is reciprocally proportional to the gap from disk or space. Inhibition, that is that the live of activity, is indicated by a zone while not growth of the organism round the disk or well. the scale of the zone relies on the speed of diffusion and growth of the organism. the foremost wide used screening strategies to live the antimicrobial effectivity of medicative plants, spice, their oil, and their constituents square measure agar diffusion methodology.[34]

With disk diffusion method, a paper disk soaked with medicinal plant extract is laid on top of an inoculated agar plate. Factors such as the volume of extract placed on the paper disk, the thickness of the agar layer and whether a solvent is used vary considerable between studies. On the other hand in agar well test is a quantitative method in which the extract is deposited into well cut into the agar and can be used with large number of extracts and/or large number of bacterial isolates (Dorman, et al). To make bacterial growth easier to visualize, triphenyl tetrazolium chloride may also added to the growth medium (Elgayyar et al., Mourey et al). Microorganisms are generally termed susceptible, intermediate, or resistance, depending upon the diameter of the inhibition zone. Quantitative results are possible with a high degree of standardization. This method should not be used for anaerobic microorganisms.

DISK DIFFUSION METHOD:

The disk diffusion methodology (also better-known the zone of inhibition methodology) is maybe the foremost wide used method as a result of its simplicity and low price. It uses solely little amounts of the check substance (10-30 µL), is completed by staff with lowest coaching (plate-1). the strategy involves the preparation of a dish containing 15-25 milliliter agar, microorganism at a better-known concentration square measure then unfold across the agar surface and allowed to determine. A paper disk (6 or eight mm) containing a better-known volume of the check substance is then placed within the center of the agar and therefore the dish incubated for twenty-four h or additional. At now the "cleared" zone (zone of inhibition) close the disk is measured and compared with zones for normal antibiotics or literature values of isolated chemicals or similar extracts. On the opposite hands, information from this assay is often conferred as mean size of zone of inhibition (with or while not normal deviation), or a ranking system like "+7kikujyhgtf", "++", and "+++" to point levels of activity. Few authors additionally offer levels of activity (slight, moderate, strong) with none relation to standardized criteria. one among the main criticisms of this methodology is that it depends on the power of the extract to diffuse through agar and any element of the extract that will diffuse far from the disk can produce a amount gradient, probably making a gradient of active medicinal drug compounds. All of the medicinal drug checking ways use associate degree binary compound base for dispersion of the test substance, either via diffusion in agar or dispersion among nutrient broth, consequently assays exploitation extracts with restricted solubility in binary compound media (e.g. essential oils) might not replicate verity medicinal drug activity. there's additionally no agreement on the simplest agar to use for these assays.

AGAR CUP METHOD:

The principle of the agar cup or agar well diffusion is that the same as that of the agar disk diffusion methodology. a regular matter culture is unfold equally on the surface of gelled gar plates. Wells of between half-dozen and eight millimeter square measure aseptically punched on the agar employing a sterile cork borer permitting a minimum of thirty millimeter between adjacent wells and therefore the dish. mounted volumes of the plant extract square measure then introduced into the wells (plate-2). The plates square measure then incubated at 37oC for twenty-four h for microorganism [35].

TURBIDIMETRIC ASSAY:

The best, most economical and productive growth measuring system is maybe the turbidimetric assay. It merely involves following the expansion of a organism with a photometer. one among the main issues with turbidimetric analysis is that the vary of detection. Spectrophotometers typically need 106 -107 CFU/ml for detection, thereby limiting cell concentrations which might be with success assayed with this methodology. this could produce a state of affairs within which no growth (i.e., no absorbance increase) is ascertained, when, in fact, undetectable growth is going on at levels below a hundred and five CFU/ml. associate degree incorrect interpretation of „lethality" may result (Davidson and Parish, 1989). There are several publications exploitation this methodology to review the antimicrobial activity of medicative plants, spices, and their essential oils.

MECHANISMS OF ANTI-MICROBIAL ACTIVITY:

The antibacterial activity of an agent is mainly ;attributed to two mechanisms, which include interfering chemically with the synthesis or function of vital components of bacteria, and/or circumventing the conventional mechanisms of antibacterial resistance. Figure 1 shows these mechanisms and as it can be observed, there are multiple targets for the antibacterial agents that comprise (I) bacterial protein biosynthesis; (II) bacterial cell-wall biosynthesis; (III) bacterial cell membrane destruction; (IV) bacterial DNA replication and repair, and (V) inhibition of a metabolic pathway. In addition, bacteria may show resistance to antibacterial agents through a variety of mechanisms. Some microorganism species square measure innately proof against one or additional categories of antimicrobial agents. In these cases, all strains of that microorganism species exhibit proof against all the members of these medication categories. a significant concern is that the microorganism acquire resistance, wherever at first inclined microorganism populations become proof against the medication agent [59]. So that, one in all the key factors find the solutions for retardation the event of antibiotic-resistant is knowing regarding the mechanisms of medication resistance [60], that chiefly embrace the activation of effluence pump, destroying the medication agents through the destruction enzymes, modification of antibiotics by the suggests that of modifying enzymes, and also the alteration of target structures within the bacteria that have lower affinity for medication recognition [61]. It ought to be conjointly noted that the resistance to medication agents are often associated with one reasonably mechanism or differing types along. the most mechanism for spreading the resistance of antibiotic through microorganism populations is plasmids within the role of genetic material, that square measure capable of being severally replicated and passed between microorganism cells and species. every of those mechanisms has been on an individual basis mentioned within the following aminoglycosides, and oxazolidinones show medication activities through this specific mechanism [62].

Proven targets for medicinal drug medicine

Supermolecule biogenesis at the cell organ is targeted by totally different categories of antibiotics like macrolides, tetracyclines, aminoglycosides. semipermeable membrane will be targeted by some antibiotics like antibiotic B. These antibiotics alter microorganism outer membrane porosity and eventually destabilize outer membrane of bacterium. The fluoroquinolone antibiotics inhibit desoxyribonucleic acid replication by trappings a posh of desoxyribonucleic acid absolute to the protein desoxyribonucleic acid Gyrase. Cell-wall biogenesis is suppressed by the varied categories of antibiotics. b Multiple antibiotic resistance mechanisms in bacterium. effluence pumps take away the antibiotics from bacterium (e.g. Fluoroquinolones and trimethoprim resistance in P. aeruginosa). Destruction enzymes that degrade the antibiotics (β-lactams in Enterobacteriaceae). Modifying enzymes that amendment the antibiotic structure (e.g. antibiotic drug or fosfomycin in P. aeruginosa)

MICROORGANISMS USED FOR ANTIMICROBIAL TESTS:

Bacterial kind strains (18 strains) used for the analysis of antimicrobial activity of all isolated epiphytes (and additionally for algae extracts) were: Aeromonas salmonicida LMG3780, A. hydrophila B3 (RVAU-Denmark), Enterococcus faecalis ATCC 29212, enteric bacteria coli O126-B16 (ATTC 14948), E. coli ATCC 25922, E. coli ATCC 8739, Micrococcus sp. (Pasteur Institute, Tunis), Pseudomonas cepacia (INSTM, Tunis), P. fluorescens AH2 (Danish Institute for Fisheries analysis, Denmark), P. aeruginosa ATCC 27853, enterobacteria typhimurium C52 (Laboratoire Hydrobiologie Marine et Continentale, Université Diamond State Montpellier II, France), cocci aureus (Pasteur Institute, Tunis), S. aureus ATCC 25923, S.

aureus ATCC 6538, Streptococcus sp. (Pasteur Institute, Tunis), true bacteria tapetis CECT4600 (Department of biology and Parasitology, University of Santiago Diamond State Compostela, Spain), V. anguillarum ATCC12964T, V. alginolyticus ATCC 17749T. additionally the yeast Candida albicans ATCC 10231 was used for the tests.

The selected most active isolate against these nineteen pathogens (the Firmicutes isolate P8) was additionally tested for antagonistic activity against nine Vibrio spp. kind strains (pathogens well delineated in marine ecosystems): V. fluvialis ATCC 33809T, V. (Aliivibrio) logei LMG14011, V.parahaemolyticus ATCC 17802 T, V. pectenicida CIP105190T, V. pectenicida LMG19642T, V. proteolyticus ATCC15338T, V. salmonicida LMG3780T, V. splendidus ATCC33125T, V. vulnificus ATCC27562T, and few alternative true bacteria species: Enterobacter xiangfangensis (CCY15226), Enterococcus durans (CCY15220), Enterococcus faecium (CCY15234), Proteus mirabilis (CCY15230), and Morganella morganii (CCY15221), originally obtained from the IFREMER Laboratory of biology of Invertebrates (Brest, France).

[ATCC: yank kind Culture assortment; CCY: Culture Collection Yerseke; LMG: Laboratory for biology Gent; RVAU: Royal Veterinary and Agricultural University (now University of Danish capital, college of Life Sciences, Denmark); INSTM: National Institute of Sciences and Marine Technologies (Salammbô, Tunisia)].

II. CONCLUSION:

This review summarizes the methods available studying the anti-microbial behavior. This information is intended to help field experts and researchers to find methods according to their needs and available resource. Some of these plants were more effective than traditional antibiotics to combat the pathogenic microorganism studied. The chance to find anti-microbial activity was more apparent in ethanol then water extract if the same plant. This plant could be source of new antibiotic components further work is needed to isolate. The secondary metabolites from the extracts studied in order to test specific antimicrobial activity.

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