Journal Homepage: -www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI:10.21474/IJAR01/5040
DOI URL: http://dx.doi.org/10.21474/IJAR01/5040
ISSN NO. 2320-5407

RESEARCH ARTICLE

ANTIMICROBIAL ACTIVITY OF DIFFERENT MEDICINAL PLANTS AND HONEY AGAINST MICROORGANISMS ISOLATED FROM INFECTED WOUND AREA ON SKIN OF HUMAN.

Megha Chevli[1], Mansi Mehta[2] and Gaurav Shah[3].

1. Student of Department of Biotechnology, Veer Narmad South Gujarat University, Surat-395007, Gujarat, India.
2. Teaching Assistant, Department of Biotechnology, Veer Narmad South Gujarat University, Surat-395007, Gujarat, India.
3. Co-ordinator, Department of Biotechnology, Veer Narmad South Gujarat University, Surat-395007, Gujarat, India.

Manuscript Info

Manuscript History

Received: 02 June 2017
Final Accepted: 04 July 2017
Published: August 2017

Key words:-
Pathogenic, Antimicrobial activity, Pharmaceutical agent, Chronic wound infection.

Abstract

The bacterial pathogens i.e. *Pseudomonas aeruginosa,Escherichia coli, and Candida famata*were isolated from infected area of human. The medicinal plant (Ardushi,Nilgiri,Ashopalav, and Ashwagandha) shows different antimicrobial activity against isolated bacteria. The best antimicrobial activity was observed in Ardusi. Alopathic medicines need to be reduced in the wound infection treatment and can be replaced with medicinal plants as a potential source of pharmaceutical agent. Honey was very promising topical antimicrobial agent against the infection of antibiotic-resistant bacteria (e.g. *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa*) and in the treatment of chronic wound infections that do not respond to antibiotic therapy. Honey is an ancient wound remedy for which there is modern evidence of efficacy in the treatment of burn wounds, but limited evidence for the effectiveness of its antibacterial activity againstPseudomonas.

Introduction:-

Ayurveda is believed to be prevalent since last 5000 years in India. It is one of the most noted systems of medicine in the world. Ayurveda is based on the hypothesis that everything in the universe is composed of five elements viz. space, air, energy, liquid and solid. Plants have played a crucial role in maintaining human health and improving the quality of human life for thousands of years. The World Health Organization has estimated that 80% of the earth's inhabitants rely on traditional medicine for their health care needs, and most of this therapy involves the use of plants extracts or their active components (Cuong, V. *et.al*., 2002).The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The beneficial medicinal effects of plant materials typically result from the secondary products present in the plant although, it is usually not attributed to a single compound but a combination of the metabolites (Sheeba, J. *et.al.*, 2012*)*.

Medicinal Uses of *Adhatodavasica* (Ardushi):Ardushi is a well-known herb in indigenous systems of medicine for its beneficial effects, particularly in bronchitis, wound infection, etc. A warm decoction of its leaves is useful in treating scabies and other skin diseases(Bhowmik D. *et.al.*, 2010). The important active components include alkaloids are vasicine and vasicinone. The former is under development as an herbal drug in India, as they are the

Corresponding Author:-Dr. Mansi Mehta.
Address:- Teaching Assistant, Department of Biotechnology, Veer Narmad South Gujarat University, Surat-395007, Gujarat, India.

semi-synthetic derivatives of alkaloids, bromhexine and ambroxol. Another property of the herb was that it helped to stop bleeding (A. Karthikeyan *et.al*., 2009).

Medicinal uses of *Eucalyptus globulus* (Nilgiri):-
Eucalyptus is one of the world's important and most widely planted genera (Sheeba, J. *et.al.*,2012*)*.These essential oils are in great demand in the market, since they find applications as anesthetic, antiseptic, astringent, deodorant, diaphoretic, disinfectant, inhalant, insect repellant, preventative, fever, flu, inflammation, malaria, mastitis, rhinitis, sore throat, spasms, trachalgia, worms, and wounds (Benali M.*et.al*., 2012).

Medicinal uses of *Saracaasoca (*Ashopalav):-
Ashoka is the most ancient tree of India, generally known as an "ashokbriksh", botanist known as a *Saracaasoca*. *Saracaasoca* is reported to contain glycoside, flavanoids, tannins and saponins. It is usedas anti-progestational, antiestrogenic activity against menorrhagia and anti-cancer(Pradhan, P. *et.al*., 2009).

Medicinal uses of *Withaniasomnifera* (Ashwagandha):-
*Withaniasomnifera*is a plant used in medicine from the time of Ayurveda, the ancient system of Indian medicine.Today there is much interest in natural products with anticancer activity. Centuries of Ayurvedic medical experience using *Withaniasomnifera* have revealed it to have pharmacological value as an adaptogen, antibiotic, aphrosidiac, astringent, antiinflammatory, diuretic, narcotic, sedative, and tonic(Sharma, P. *et.al.*, 2010).

Medicinal uses of Honey:-
The antimicrobial activity of honey is attributed largely to osmolarity, pH, hydrogen peroxide production and the presence of other phytochemical components e.g. methylglyoxal.Honey is an ancient wound remedy for which there is modern evidence of efficacy in the treatment of burn wounds, but limited evidence for the effectiveness of its antibacterial activity against *Pseudomonas*(Cooper, R. *et.al*., 2002).

Human wound infection:-
Wound is defined as loss or breaking of cellular and anatomic or functional continuity of living tissue due to physical, chemical, thermal, microbial or immunological exploitation to the tissues .Wound healing is complex multistep physiological process that involves multitude of cells and events. It is a dynamic, interactive process which involves soluble mediators, blood cells, extracellular matrix and parenchymal cells (Saini, S. *et.al.,* 2015),. *Pseudomonas aeruginosa* also causes nosocomial infections as a result of its ubiquitous nature, ability to survive in moist environments and resistance to many antibiotics and antiseptics. It is obligate aerobe, motile, rod shaped, and measuring about 0.6 x 2 μm. It is gram-negative and occurs as single bacteria, in pairs, and occasionally in short chains (Nascimento, G.*et.al*.,).*Escherichia coli* are gram-negative, non-sporing bacilli with most strains being motile and generally possessing both sex pili and adhesive fimbriae. *Escherichia coli* was initially considered a non-harmful member of the colon flora, but is now associated with a wide range of diseases and infections including meningeal, gastrointestinal, urinary tract, wound and bacteremia infections in all age groups (Saini, S. *et.al.*, 2015).*Candida famata* (also known as *Debaryomyces hansenii* and *Torulopsis candida) is* commensal *yeast* found in cheese, dairy products and the environment it has been described in human infections, including catheter-related bloodstream infections, peritonitis, acute zonal occult retinopathy and mediastinitis. It is a rare cause of candidiasis, accounting for only 0.2%–2% of isolates collected from antifungal surveillance studies.

Materials and Methods:-

Collection of Sample:-
Human wound infection swab sample was collected from Microcare Laboratory, Surat, Gujarat, India in sterile container/condition and preserved in refrigerator (20°C) till it was used for the isolation of microorganisms in laboratory.

Isolation of Microorganisms:-
Collected sample swab stick was streaked onto the Blood agar, MacConkey's agar, and Nutrient agar plate. After streaking, plates were incubated at 37°C for 48 h.

Identification of microorganisms:-
Identification of isolated microorganisms were performed according to morphological, cultural and biochemical characteristics. All isolated microorganisms were subjected to Gram's staining,biochemical tests and by VITEK Analysis. Identification of microorganisms by following ways:
Colony morphology: Colonial morphology was done to determine the morphology of selected strains on the basis of size, shape, and colour of bacterial colony.
Biochemical test:
1. Carbohydrate fermentation (Sugar utilization) test
2. Methyl red (M-R) test
3. Voges – Proskauer(V-P) test
4. Citrate utilization test
5. Indole production test
6. Triple sugar iron (TSI) test
7. Lead acetate paper – strip test

Collection of plants material and Honey:-
Different plant material was collected from campus of V.N.S.G.U., Surat. Leaves of such a medicinal plant like, Ashopalav (*Saracaasoca*), Nilgiri (Eucalyptus *globulus*), Ardushi (*Adhatodavasica*) were collected from Botanical Research Centre (bapalal), V.N.S.G.U., Surat to check its antimicrobial activity against isolated organisms. And another two sample i.e. powder of Ashwagandha (*Withaniasomnifera)* was collected from shop Annapurna, Bhatar, Surat and honey of Dabur company was collected for check its antimicrobial activity against isolated microorganism.

Plant extracts preparation:-
Collected Plant material were (for example, a plant leaf of Ardushi, Nilgiri, Ashopalav) dried at 40°Cfor overnight. It was needed to be crushed, using a pestle and mortar, to provide a greater surface area. After the crushing, the plant material was sufficient to fill the porous cellulose thimble for soxhlet extraction.

Extraction by Soxhlet method:-
The solvent (250 mL of Methanol) was added to a round bottom flask, which was attached to a Soxhlet extractor and condenser on heating mantle. The crushed plant material (10 g Ardushi, 15 g Nilgiri and 8 g Ashwagandha) was loaded into the thimble, which was placed inside the Soxhlet extractor. The side arm was lagged with glass wool. The solvent was heated using the heating mantle and would begin to evaporate, and then moving through the apparatus to the condenser. The condensate then dripped into the reservoir containing the thimble. Once the level of solvent reached the siphon it poured back into the flask and the cycle begins again. The process should run till solvent look clear appearance. After soxhlet extraction collect extracted material into pre weigh flask for compete evaporation of solvent. After evaporation dry mass was collected and dissolve into DMSO (Dimethylsulphoxide) as dissolving agent. Prepared plant extract was use for antimicrobial activity (Redferm, J.*et.al*., 2014).

Powder of ashwagandha (10 g) was mixed into 125 mL of methanol and kept it into rotatory shaking incubator for 48 h at 30°C. After that it was also collected in reweight beaker and was kept it in oven for complete evaporation of methanol. After evaporation it was dissolved into dissolving agent DMSO.

Antimicrobial activity:-
The antimicrobial assay was performed by Agar well diffusion method for plant extract. The media (Mueller Hinton Agar) was poured into the Petri plate. The isolated microorganisms were cultured in to Mueller Hilton Agar by using spread plate technique and a well of 7mm diameter was made onto the plate for loading the extract. Stock solution in DMSO was used to prepare desired dilutions with different concentration. Each dilutions was poured in to well and the sample extracts were allowed to diffuse properly by keeping the petri plates in refrigerator at 4°C for 30 min followed by the incubation at 37°C for 24 h. Solvent used for extraction was used as a control in the same manner. The diameter of zone of inhibition (excluding well diameter) was taken as the measure of the antimicrobial activity of a particular extract (Pawar, P. *et.al*., 2009).

Result and Discussion:-
Isolation of unknown microorganism by Gram's staining revealed following result (Table: 1).

Vitek Analysis:-
Unknown bacterial cultures were submitted to Advance Diagnostic Laboratory, Surat for identification. According to VITEK result strain A and A1 were confirmed that *Escherichia coli*, strain C was confirmed *Pseudomonas aeuruginosa* and C1 was *candida famata* (Table: 2).

Plate:-1 Isolation of microorganism on MacConkey's Agar Plate.

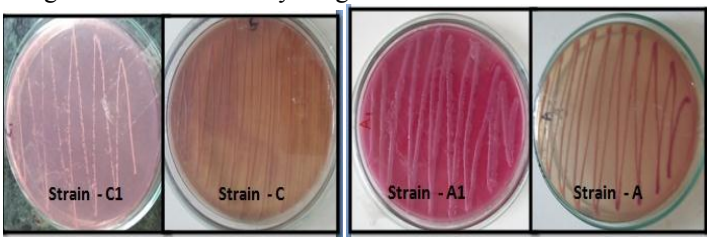

Table1:- Result of Gram's Staining.

Strain	Characteristics
A (*Escherichia coli*)	Gram -ve
A1 (*Escherichia coli*)	Gram -ve
C (*Pseudomonas aeruginosa)*	Gram -ve
C1 (*Candida famata*)	Gram -ve

Table 2:-Name of identifying microorganisms.

Strain of microorganism	Name of organism
A	*Escherichia coli*
A1	*Escherichia coli*
C	*Pseudomonas aeruginosa*
C1	*Candida famata*

Table3:-Stock concentration of plant extracts.

Extract	Concentration of stock (mg/mL)
Eucalyptus globulus	5.5g/15mL DMSO
Saraca asoca	0.6g/4mL DMSO
Adhatoda vasica	2.783g/10mL DMSO
Withania somnifera	1g/5mL DMSO

Plant extract of Ardushi, Nilgiri, and Ashopalav were obtained by soxhlet by using methanol as solvent (200mL). After plant extract preparation by soxhlet method, extracted material was kept for evaporation of solvent (Methanol) at room temperature for overnight, then after weight it and dissolve dry mass into DMSO (Stock concentration and different dilutions of plant extract shown in Table: 3 and Table: 4).

Table 4:-Different concentration of plant extract.

Extract	Stock	1	2	3	4	5
	Concentration g/mL DMSO					
Eucalyptus globulus	5.5/15	0.2/2	0.25/2	0.22/2	0.18/2	0.14/2
Saraca asoca	0.6/4	0.12/2	0.105/2	0.09/2	0.075/2	0.06/2
Adhatoda vasica	2.783/10	0.22/2	0.194/2	0.166/2	0.139/2	0.111/2
Withania somnifera	1.0/5	0.16/2	0.14/2	0.12/2	0.10/2	0.008/2

Table 5:-Results of Antimicrobial activities of different Medicinal plants and Honey.

Code	Name of Organism	Name of Plant Sample	Concentration of Stock Solution	Diameter of zone of inhibition (mm)					
				Stock	1	2	3	4	5
A	*E. coli*	*Eucalyptus globulus*	5.5g/15mL DMSO	-	-	-	-	-	-
A1	*E. coli*			-	-	-	-	-	-
C	*P. aeruginosa*			6	4	3	1	1	-
C1	*C. famata*			8	7	5	5	6	3
A	*E. coli*	*Saraca asoca*	0.6g/4mL DMSO	-	-	-	-	-	-
A1	*E. coli*			-	-	-	-	-	-
C	*P. aeruginosa*			5	4	3	1	-	-
C1	*C. famata*			11	5	3	3	2	1
A	*E. coli*	*Adhatoda vasica*	2.78g/10mL DMSO	11	10	6	5	3	-
A1	*E. coli*			8	5	4	1	-	-
C	*P. aeruginosa*			-	-	-	-	-	-
C1	*C. famata*			5	5	3	1	3	3
A	*E. coli*	Honey	5mL	5	1	-	-	-	-
A1	*E. coli*			-	-	-	-	-	-
C	*P. aeruginosa*			-	-	-	-	-	-
C1	*C. famata*			3	2	-	-	-	-
A	*E. coli*	*Withania somnifera*	1g/5mL DMSO	-	-	-	-	-	-
A1	*E. coli*			-	-	-	-	-	-
C	*P. aeruginosa*			7	4	3	1	-	-
C1	*C. famata*			13	10	-	-	-	-

The human bacterial pathogens i.e. *Pseudomonas aeruginosa, Escherichia coli, Candida famata* were isolated from Human wound infected swab. The isolated bacteria are highly pathogenic for human. The medicinal plant (Ardushi, Ashopalav,Ashwagandha, Nilgiri and Honey) showed different antimicrobial activity against isolated bacteria. The best antimicrobial activity is observed in Ardusi was 11mm diameter of zone of inhibition (Table: 5).

Conclusion:-

Plants have played a crucial role in maintaining human health and improving the quality of human life for thousands of years. Medicinal plants were used for treating major or minor diseases of human. So main purpose of carrying out this experiment was some medicinal plant like Ashopalav, Amla, Nilgiri, Ashwagandha, Ardushi, Neem, Satavari, were used as medicine. Pathogen was resist against modern or allopathy medicines and antibiotics also show adverse effects, so we need concern about this. To overcome these problem people starts work on preparing a medicine from plant material sources. Clinical microbiologists have main reasons to be interested in the topic of antimicrobial plant extracts that is phytochemicals will find their way into the arsenal of antimicrobial drugs and another reason is that public is becoming increasingly aware of problems with the over prescription and misuse of traditional antibiotics. Plant compounds have no any side effects so people start use. Honey made it a very promising topical antimicrobial agent against the infection of antibiotic-resistant bacteria (e.g. *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa*) and in the treatment of chronic wound infections that do not respond to antibiotic therapy.

References:-

1. A. Karthikeyan*et.al*. (2009), "Preliminary phytochemical and antibacterial screening of crude extract of the leaf of *Adhatodavasica*" *International journal of green pharmacy.*
2. Benali M. *et.al*. (2012), "Antimicrobial activity of essential oil from the leaves of *Eucalyptus globules* against *Escherichia coli* and *Staphylococcus aureus"Asian Pacific journal of tropical biomedicine*, Vol. **2(9)**, pp. 730 – 742.
3. Cooper, R. and Halas, E. (2002), "The efficiency of Honey in inhibitory strain of *Pseudomonas aeruginosa* from infected burns" *Journal of burn care & rehabilitation*, Vol. **23**, pp. 366 – 370.
4. Cuong, V. and Michael, O. (2002), *Journal of microbiology infections*, Vol. **4**, pp. 481 – 489.
5. DesalegnAmenu. (2014). "Antimicrobial activity of medicinal plant extracts & their synergistic effect on some selected pathogens" *American journal of Ethnomedicine*, Vol. **1**, pp. 018 – 029.
6. Kumar, Bhowmik D. and Tiwwari, P. (2010), "Indian traditional herbs *Adhatodavasivca*& its medicinal application" *Journal of chemical and pharmaceutical Research*, Vol. **2**, pp. 240 – 245.
7. Nascimento, G.*et.al*. "Antimicrobial activity of plant extracts and phytochemicals on antibiotic resistant bacteria" *Brazilian journal of microbiology*, Vol. **31**, pp. 247 – 256.
8. P. Saranraj and S. Sivasakthii. (2014), "Medicinal plants and its antimicrobial properties: A review" *Global journal of Pharmacology*, Vol. **8(3)**, pp. 316 – 327.
9. Patel, R. (2009) "Experimental Microbiology" Vol. **1**Fifth Edition.
10. Pawar, P. *et.al*. (2009), "Antimicrobial activity of some crude herbal drugs used for skin diseases by pawra tribes of nandurbar district" *Indian journal of natural products and resources*, Vol. **1(2)**, pp. 216 – 220.
11. Pradhan, P. *et.al*. (2009), "*Saracaasoca* (Ashoka): A review" *Journal of chemical & pharmaceutical research*,Vol. **1(1)**, pp. 62 – 71.
12. Rajput, K. "Common medicinal plants".
13. Redferm, J.*et.al*. (2014), "Using soxhlet extraction to produce and test plant material (essential oil) for their antimicrobial properties" *Journal of microbiology and biology education*, Vol. **15(1)**, pp. 45 – 46.
14. Rios, J. and Recio, M. (2005), "Medicinal plants and antimicrobial activity" *Journal of Ethnopharmacology*, pp. 80 – 84.
15. Saini, S. *et.al.* (2015), "Traditional Indian medicinal plants with potential wound healing activity: A review" *International Journal of Pharmaceutical Science and Research*.
16. Sharma, P. *et.al.* (2010), "Biological activity of *Withaniasomnifera*" *Annals of biological research*, Vol. **1(3)**.
17. Sheeba, J. and Selva M. (2012), "Antimicrobial activity of *Adhatodavasica* against clinical pathogen" *Asian journal of plant sacience& research*, Vol. **2(2)**, pp. 33 – 83.