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Antimicrobial Activities from the Essential Oil from Leaves of Eucalyptus Globulus Against the Microbes Isolated from Contaminated Soil, Water & Milk

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Abstract - Eucalyptus species are well known as medicinal plants because their biological and pharmacological properties. In the international pharmacopeia, the most important and represented species, used anaesthetic, anodyne, antiseptic, astringent, deodorant, diaphoretic, disinfectant, expectorant, febrifuge, fumigant, haemostat, inhaltant, insect repellent, preventive, rubefacient, sedative yet abscess, arthritis, asthma, bolus, bronchitis, burns, cancer, diabetes, diarrhea, diphtheria, dysentery, encephalitis, enteritis, erysipelas, fever, flu, inflammation, laryngalgia, laryngitis, leprosy, malaria, mastitis, trachalgio, worms and wounds, some time their demand is also high in the soap and cosmetic industries.

Keywords:- Antimicrobial, Essential oils, Eucalyptus leaves, Isolated microbes, Cancer activities.

INTRODUCTION

Eucalyptus globulus belongs to the Myrtaceae family, native to Australia (Motaetal., 2015).It is tall evergreen tree,it was introduce in Algeria in 1854 by Rame(Bachir Raho & Benali, 1999). This oil has been used especially to manufacture pharmaceutical inhalants,nasal discharge stimulants, oral care products, or even with the function of flavor and aroma to medicines. However, recent evidence points to possible effects associated with healing, anti inflammatory and antimicrobial action(Mota et al., 2015).Eucalyptus species are well known as medicinal plant because of their biological and pharmacological properties.In the international pharmacopeia,the most important and represented species, used aanesthetic,anodyne, antiseptic, astringent, deodorant, diaphoretic, disinfectant,expectorant, febriu-, fumigant, hemostal, inhaltant, insect repillant, preventive, rubefacient, sedative yet abscess, arthristic, asthma, boils, bronchitis, burns, cancer, diabetes, diarrhea, diahtheria, diphtheria, dysentery, encephthalitis, enteritis, erysipelas, fever, flu, inflammation, laryngalgia, laryngitis, lep-orosy, malaria, mastitis, miasma, phasms, trachalgio, worms, and wounds, some time their demand is also high in the soap and cosmetic industries(Bachir Raho,2012).

Leaf extracts of Eucalyptus have been recognized as natural food preservatives (Mori, 2004).All aforementioned products are widely cultivated in Brazil and evaluating the potential of these substances can result in future investigations hypotheses. For example, the development of antiseptic or disinfectant products to address the emerging risk of reduction in sensitivity of the microorganisms to the germicidal agents. Another example is to stimulate discoveries related to the prevention and treatment of bio-films that slow or prevent the healing of wounds (especially in chronic wounds), generating high costs for treatment and associated morbidity and mortality. Therefore, the present study sought to evaluate the in vitroantimicrobial activity of the essential oil of Eucalyptus globulus, of xylitol against the following microorganisms: Pseudomonas aureginosa; Samonella sp.; Staphylococus aureus; Proteus vulgaris; Escherichia coli and Candida albicans. (Valéria de Siqueira Mota:etal). Reportshave shown that about 50,000 people die in the world per daydue to infectious diseases mainly caused by Candida albicans, Escherichia coli, Pseudomonas aeruginosa and Staphylococcusaureus. (Muhammad Rifaqat Ammer:eta).Eucalyptus (EO)has antibacterial,antiviral,antifugal components and a long history of use against the effect of colds, influenza, other respiratory infection, rhinitis, and sinusitis(Angela E.sadlon:etal). Escherichia coli, originally called “Bacterium coli” was first isolated from the faces of achild in 1885 by the Austrian pediatrician TheodorEscherich (Escherich, 1885). Escherichiacoli is a common inhabitant of the gastrointestinaltract of humans and animals. There are E.Colistrains that are harmless in theintestinal tract. The pathogenic E.coli are divided into those strains causing diseaseinside the intestinal tract and others capable ofinfection at extra-intestinal sites (Kaper et al., 2004). Escherichia colican be found secondarily in soil and water asthe result of fecal contamination, E. coli may freely replicate intropical fresh water (Bermudez and Hazen, 1988).Classically, itsdetection has been used as an indicator of poorwater quality

(Rodney A. Welch). The ability to build a phylogeny forsome of the E. coli pathotypes is complicatedbecause the horizontally acquired islands do notalways share chromosomal location and geneticcontent (Welch et al., 2002). Escherichia coli are Gram-negative, nonsporeformingbacilli. They are approximately 0.5µmin diameter and 1.0–3.0µm in length. Within theperiplasm is a single layer of peptidoglycan, peptidoglycan has a typical subunit structurewhere the N-acetylmuramic acid is linked by anamide bond to a peptide consisting of L-alanine,D-glutamic acid, meso-diaminopimelic acid andfinally D-alanine.Escherichia coli are commonly motile in liquid by means of peritrichous flagella. Swarmingbehavior and differentiation into hyperflagellatedand elongated bacilli typical of that seenwith the Proteus species can be observed onsome solid media (Harshey 1994). Most E. coli strains are capable of growingover a wide range in temperature (approximately15–48°C). The growth rate is maximal inthe narrow range of 37–42° (C Ingraham andMarr, 1987). Escherichia coli can grow within apH range of approximately 5.5–8.0 with bestgrowth occurring at neutrality. Some diarrheagenicE. coli strains have the ability to tolerateexposure to pH 2.0. Such an acid shock mimicstransit through the stomach and induces expressionof sets of genes involved in survival andpathogenesis (Waterman and Small, 1996).Escherichia coli provide many necessary vitamins including Vitamin K and B-complex vitamins. Wehave billions of E.coli bacteria in our bodies, making things we need, helping digest our food and maintainingour health. Although most E. coli are harmless and are a needed bacterium for health, there are some strains of E.Colibacteria that can be very harmful to our health. A rare strain of E. coli that you may have seen in the newscan cause potentially dangerous outbreaks and illness. This strain is E. coli O157:H7. This E. coli canproduce a toxin called Shiga-like toxin (SLT).The harmful strain of E. coli bacteria can cause abdominal cramping, diarrhoea and occasionally vomiting.Usually little or no fever is present. Dehydration, even in mild cases of diarrhoea, can easily occur. Normallythe illness resolves in 5 to 10 days. In 5%-10% of cases, hemolytic uremic syndrome (HUS), which ischaracterized by kidney failure and loss of red blood cells, can occur. In severe cases of the disease, 2%-7% may have permanent kidney damage. Dehydration is particularly dangerous to small childrenwho are too small to tolerate much blood and fluid loss. The presence of these bacteria can also be verydangerous to the elderly population or persons who are already ill(Centers for Disease Control and Prevention, National Center for Infectious Diseases, Division of Bacterial andMycotic Diseases. January 27, 2004).

Staphylococcus aureus isGram-positive, Cocci in grape-like clusters(Sarkis K. Mazmanian, 7 FEBRUARY 2003).Staphylococcus aureus is one of the most commoncauses of community- and health care–associatedinfections, Staphylococcus aureus is one of the most successful human pathogens, little is known about its effects on the U.S.population as a whole.We analyzednewlyavailable datafrom the 2001–2002 National Health and Nutrition ExaminationSurvey (NHANES).(Philip L. Graham III, 7 March 2006). Staphylococcus aureus accounts for about 13% of allnosocomial blood infections, and is the second mostcommon cause of these infections after coagulasenegativestaphylococci.1 S.aureus bacteraemia extendslength of hospital stay and increases antibiotic use, costs,and mortality.1 Nasal carriage is a risk factor for acquiringnosocomial infection.2 haveshown that 80% of nosocomial S. aureus bacteraemiaepisodes in carriers of the bacteria are attributable to anendogenous source.(Heiman F L Wertheim,elat Vol 364 August 21, 2004). The gram positive bacterium S.aureus is mainly responsible for postoperative wound infection .Expression of virulence determinants in S. aureu.It is known for its capacity to cause a broad range of important infections in humans. Such capacity is related to the expression of an array of factors that participate in pathogenesis of infection, allowing this bacterium to adhere to surfaces/tissues, avoid or invade the immune system, and cause harmful toxic effects to the host. These factors are known as virulence determinants (Table 1), and can be divided into cell-surface-associated (adherence) and secreted (exotoxins) factors.Table 1 Virulence factors involved in the pathogenesis of Staphylococcus aureus and respective putative function.(Ana Rita Costa. 2013).

Table-1

VIRULENCE FACTOR	PUTATIVE FUNCTION
CELL SURFACE FACTORS Microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) Staphylococcal protein A (SpA)	Bind to IgG, interfering with opsinization and phagocytosis
Fibronectin-binding proteins (FnbpA and FnbpB)	Attachment to fibronectin and plasma clot
Collagen-binding protein	Adherence to collagenous tissues and Cartilage
Clumping factor proteins (ClfA and ClfB)	Mediate clumping and adherence to fibrinogen in the presence of fibronectin
SECRETED FACTORS Superantigens Staphylococcal enterotoxins (SEA, B, C, D, E, G and Q)	Massive activation of T cells and antibody presenting cells
Toxic shock syndrome toxin-1 (TSST-1)	Massive activation of T cells and antibody presenting cells
Cytolytic toxins	
Cytolysins	Induce lysis on a wide spectrum of cells, mainly platelets and monocytes
α-hemolysin	
β-hemolysin	Hydrolysis of sphingomyelin of the plasmatic membrane of monocytes, erythrocytes, neutrophils and lymphocytes; make cells susceptible to other lytic agents
γ-hemolysin	Induce lysis on erythrocytes and Leukocytes
Leukocidin family Leukocidins E/D and M/F-PV	Induce lysis on leukocytes Induce lysis on leukocytes
Various exoenzymes Lipases	Inactivate fatty acid Cleave nucleic acids
Nucleases	Inactivate neutrophil activity; activate T cells (only ETA and ETB)

Proteases	
Serine (e.g. exfoliative toxins ETA and ETB)	Block neutrophil activation and Chemotaxis
Cysteine (e.g. staphopain)	Inactivate antimicrobial peptides
	Degrade hyaluronic acid
Aureolysin	Activate plasminogen; inactivate antimicrobial peptides
Hyaluronidase	
Staphylokinase (SAK)	Inhibit complement activation
	Inhibit complement activation
Miscellaneous proteins	Inhibit chemotaxis and activation of Neutrophils
Staphylococcal complement inhibitor (SCIN)	Inhibit chemotaxis of neutrophils
Extracellular fibrinogen binding protein (Efb)	
Chemotaxis inhibitory protein of S. aureus (CHIPS)	Inhibit neutrophil migration
Formyl peptide receptor-like 1 inhibitory protein (FLIPr)	
Extracellular adherence protein (Eap)	

2. MATERIAL

The Eucalyptus leaves were collected from rural area Debagarh distric and from urban area Bhubaneswara. It was crush manually with wooden arrangement and made it small pieces. The bacterial sample were collected from 3 different places (i) drain water collected from drain of AMIT,(ii) soil collected from near the dustbin, (iii)the unpasteurized raw milk were collected from cattle house of Khordha.

11 2 (fig- Eucalyptus leaves were collected from 1.rural area and 2.urban area)

3.METHOD

Extraction of oil was carried out by Suxhlet apparatus and it was working on steam distillation process.20gm pieces of leaves was taken and 200ml distilled water were used and working temperature is maintained at 100 degree Celsius.

(fig-Suxhlet apparatus)

and distilled for 1 hours. Once the distillation started the sample started boiling and vapour are formed, the vapour was cooled down with the help of condensed. The condensed material was collected on the other side of setup. The collected material is mixture of oil and plant extract. After the water was separated by the rotary evaporator. The Eucalyptus oil was purified which used as antimicrobial material.

Confirmation test of presence of oil in the plant extract by grease paper test. Two piece of filter paper were taken, then add drop of rural area oil on fist paper and urban area oil on another paper piece. The paper did not absorb the thick plant extraction which prove the presence of oil

Confirmation test fir presence of oil in the plant extract by chemical method, a few drop of Phenopthalin was taken in attest tube then added two drop of alkali solution (NaOH) and its colour was converted into pink colour

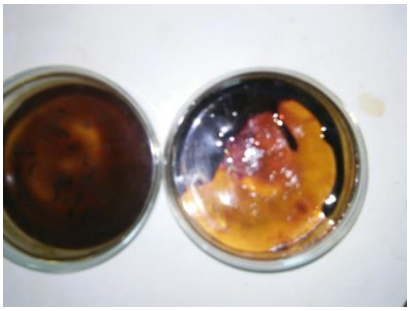
Then the plant extracted oil was added on the pink colour solution and its colour converted into white which indicated the presence of fatty acid. The extracted oil was kept in 4 degree Celsiusfreezer. Then 2.8gm of nutrient agar was dissolved in 200 ml of distilled water and 2.6gm of nutrient broth was dissolved in another 200ml of distilled water and both solution were closed with cotton block and to autoclave at 121 degree Celsius. The will sterilized nutrient agar was transformed into 3 plates and allow incubation for 24 hours. Then after 24 hours nutrient broth transformed in to 3 test tubes then the microbesculture were transformed into the nutrient broth and allow them to incubation for 24 hours.

For gram staining,after 24 hours the microbes culture inoculate in a sterilized slide was heat fixed and an staining try ,then the smear was wetted with crystal violet gently and allowed to stand for 1 minute, then the slide was slightly tilled and gently rinsed with tap water using a wish bottle, then the smear was gently flooded with Gram's iodine and again allowed to stand for one minute,the slide was slightly tilled and gently rinsed with tap water, the smear appear as a purple circle on the slide, then 95% ethyl alcohol was used to decolourize, the slide was slightly tilled until the alcohol run almost clear. the slid was immediately rinsed, then it was gently flooded with safranine to stand for 45 second. Then slid was gently rinsed with tap water, then slid was dried with bibulous paper, then the smear was viewed using a light microscope under oil immersion.

For the antimicrobial screening, numbers of small rounded filter paper were taken and wetted with plant extracted oil and inoculated in to the freshly made nutrient agar plates in which the respective microbes were inoculated for grown.

4. RESULT

Result of oil extraction



12(fig-plant extraction from 1.rural 2.urban)

Result of grease paper test

(fig- grease paper test) . The paper did not absorb the thick plant extraction which prove the presence of grease.

Result of plant extract by chemical method

(fig-before addition of plant extraction oil)

(fig- after addition of plant extraction oil)

We found that the plant extraction is a fatty aide.

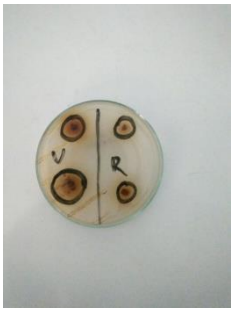
Result of microorganisms grow

(fig- microorganisms grow in nutrient agar)

Result of gram staining

No. of slide sample taken	Colour observation	Shape	Character
1.Drain water	Purple	Round	Gram +ve
2. Drain water	Purple	Round	Gram +ve
3.soil	Pink	Rod	Gram –ve
4. soil	Pink	Rod	Gram –ve
5.milk	Purple	Round	Gram +ve
6. milk	Purple	Round	Gram +ve

Result of antimicrobial screening



(fig- antimicrobial screening)

Rain water

sample	Zone in cm
Rural area	1
Urban area	1.5

Milk sample

Rural area	0.8
Urban area	1.8

Soil sample

Rural area	1.8
Urban area	1

From the experiment we found that plant extraction is acted as antimicrobial oil.

5.CONCLUSIONS

The addition of essential oil leaves in broth culture inoculated with S. aureus and E. coli inhibited the growth of these organisms. The rate of inhibition was greater, on gram negative bacteria (E. coli) than that observed on gram positive bacterium (S. aureus). In most cases the size of inoculum and the concentration of essential oil leaves affected the growth/survival of the organisms.The growths of tested bacteria in high concentrations of essential oil leaves were highly inhibited, where it was considered that these organisms were sensitive to the oil.Some authors have reported that gram-negative microorganisms are slightly more sensitive to essential oils when compared to gram-positive. The gram-positive andgram-negative microorganisms differ in several aspectsother than with respect to the structure of their cellularwalls, mainly with regard to the presence of lipoproteins andlipopolysaccharides in gram-negative bacteria that form abarrier to hydrophobic compounds. Some researchers reported that there is a relationship between the chemical structures of the most abundant in the tested essential oil and the antimicrobial activity.The antibacterial activity of Eucalyptus extracts has been due to the components such as 1,8-cineole, citronellal,citronellol, citronellyl acetate, p-cymene, eucamalol,

limonene, linalool, β- pinene, γ- terpinene, α- terpinol,alloocimene and aromadendrene .The essential oils from the leaf of E. globulus showedvarying degrees of antibacterial activity against two clinicalisolates. From the above experiment it can be inferredthat extract suggest significant growth inhibiting effectson Gram-positive (E. coli) and Gram-negative bacteria (S. aureus). The efficacy of leaf oil of E. globulus against thesemicroorganisms may provide a scientific ground for theapplication of the herb in the prevention and treatment ofbacterial infections caused by various pathogenic bacteriasuch as

Staphylococcus aureus and Escherichia coli, whichhave developed resistance to antibiotics. The incorporationof this oil into the drug formulations is also recommended.

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