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COMPARATIVE STUDY ON THE ANTIMICROBIAL PROPERTIES OF LEAF AND LEAF FIBER EXTRACT OF ANANUS COMOSUS AND AGAVA CANTALA

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Abstract - *Plants have been used for thousands of years to deal several microbial infections. Almost all the parts of the plant such as stem, leaves, roots, flowers have been used for their antimicrobial potential. Fibers which form a phase of the plant skeleton play a vital role in our lives, not only in moral clothing and also in different textiles, but in unexpected areas such as the microbial industry. They are good for the environment. Cotton, wool, silk and other plant and animal fibers are sustainable resource, as they are renewable, biodegradable and they can be used without depleting or damaging the environment. With this background, the present study has been taken up to evaluate the antimicrobial activity of extracts of the leaves and leaf fiber of two commonly known medicinal plants- agava cantala and ananas comosus (pineapple). The chemical retting method was applied to extract the fibers from plant leaves. The methanolic extracts of the leaf pulp and leaf fiber of above mentioned plants were prepared by using soxhlet apparatus. The antimicrobial activity of these extracts was evaluated by using inhibition zone diameter i.e., agar well diffusion method, disc diffusion method and dilution method using Gram negative and positive bacteria and also fungi. The organisms being tested in the present study are Malassezia furfur, Aspergillus Niger, Staphylococcus aureus and Pseudomonas aeruginosa. All of the leaf pulp extracts have shown potent antimicrobial activity and the extract of agava cantala have shown the most potent activity compare to others leaves and fibers. The study confirmed that Agava Cantala, Ananas comosus leaves possess inhibitory properties as a consequence can serve as an choice therapy for wounds and positive pores and skin infections.*

***Key Words*: Agave Cantala, Ananas Comosus, Retting, Soxhlet, Antimicrobial activity, Malassezia furfur, Aspergillus Niger, Staphylococcus aureus, Pseudomonas aeruginosa**

1. INTRODUCTION

Plant kingdom has been a major source of useful substances for the human health [1] and important advances in the knowledge of the chemical composition of a great deal of plant species have been got [2-4] .However, a great deal of plant species waits yet to be the aim of phytochemical studies.

The plant compounds are among the most studied secondary metabolites, their broad distribution in plant kingdom, great chemical diversity, and wide spectrum of biological activities, some with important impact on mammalian biology, making them attractive for phytochemical studies. The screening of such compounds of plant extracts may reveal the diversity, the abundance, and the distribution of those important compounds in the plant kingdom, and as well may facilitate the identification of alternative sources of natural products with biological activities. The exploration of the phenolic compounds accumulated by the vast plant species worldwide and the creation of databases systematizing the information about the distribution, variability, and biological properties of those compounds are among the major issues to advance in the understanding of the use and conservation of plants as source of beneficial chemical compounds for the human being. Few species of the Agave have been studied for their phenol composition and related biological activities, but the results of the studies before done suggest that an important richness of biologically active compounds exists in the Agava cantala and *Ananas comosus (pine apple)*. The relevance and the efforts already made are worthy for systematizing the information in order to facilitate sustainable further studies. That was the aim of the present paper.

1.1 Agava cantala

It is a tropical plant having lance-shaped, thorn-edged leaves growing directly from the stalk to form a dense rosette. The fiber is freed from the leaves by mechanical decortications, a scraping or peeling operation, or by a retting process common in the Philippines, employing saltwater and producing fairly weak and stained fiber. The fiber strands, white in color, are 75 to 150 cm (30 to 60 inches) long, of fine diameter, and moisture-absorbent.

Animicrobial activity of Agave cantala is reported by several workers against various gram positive, gram negative bacteria and fungus Staphylococcus aureus, Salmonella typhi, Escherichia coli, Streptococcus pyogenes, Candida albicans, B. cereus, M. luteus, P. aureginosa, S. cholereasuis, C. albicans Shigella dysenteriae, Bacillus atrophaeus, Enterococcus faecalis, Pseudomonas aeruginosa, Candida albicans, Bacillusm stearo thermophilus [5][6][7][8] . Agave cantala was found tremendously potent antimicrobial agent against various species

1.2 Ananas comosus (pineapple)

Natural fiber has been an important textile material in human civilization. The fabrics of pineapple leaf fiber are easy to print and dye, sweat-absorbent and breathable, hard and not wrinkling, and it has good antibacterial and deodorization performances. PALF has a good potential as reinforcement in thermoplastic composite. Pineapple Leaf Fiber (PALF) serving as reinforcement fiber in most of the plastic matrix has shown its significant role as it is cheap, exhibiting superior properties when compared to other natural fiber as well as encouraging agriculture-based economy. Assam has a rich source of nature's support with the cheap labor cost for the extraction purposes. The medium scale production of PALF can be increased with the proper resource utilization.

Various parts of the plant pineapple (Ananas comosus) are used in traditional medicine worldwide for treatment of a number of diseases and disorders. In folk medicine, pineapple leaf extract was used as an antimicrobial, vermicide, purgative, emmenagoogue, abortifacient, anti-oedema and anti-inflammatory agent. Compared to the fruit and stem extracts of pineapple, information about its leaf extract is limited. The potential of pineapple crown leaf extract as an ethno-medicine has been evaluated in terms of its enzymatic activities related to wound healing, antimicrobial property and toxicity. Pineapple leaf extract is nontoxic, contains enzymes related to damage tissue repairing, wound healing and possibly prevents secondary infections from microbial organisms.

1.3 MALASSEZIA FURFUR:

M.*furfur* is a common agent on human skin but under certain conditions becomes pathogenic. Environmental conditions such as increased temperature or humidity can increase the volume of skin secretion, which in tum may stimulate M.*furfur* growth. Application of oily substances, such as baby oil, onto skin may also contribute to a growth increase of M furfur. Increased sweating due to stress may also play a role in M.*furfur*. This fungus infects any age group regardless of race but adolescents have been found to have the highest rate of skin colonisation when the sebaceous glands become more active and the concentrations of lipid secretion increases [9]. Skin infections caused by M.*furfur* are dandruff, pityriasis versicolor, Malassezia folliculitits and seborrhoeic dermatitis. These conditions are not thought to be transferable or contagious.

The colonies of M.*furfur* are shiny and white to cream later becoming dull and beige, resembling bacteria-like colonies. Growth takes around one to two weeks on modified SDA which must be supplemented with fatty acids (usually done by covering medium with a thin layer of olive oil) at a temperature range of 20-35^0 C.

1.4. ASPERGILLUS NIGER:

Aspergillus *niger* is a haploid filamentous fungus and is a very essential microorganism in the field of biology. In addition to producing extracellular enzymes and citric acid, A. niger is used for waste management and biotransformation. It is usually found in common mesophilic environments such as soil, plants, and enclosed air environments. A. niger is not only a xerophilic fungi (mold that doesn't require free water for growth, can grow in humid environments), but is also a thermo tolerant. Because of this property, the filamentous fungus exhibits a high tolerance to freezing temperatures. A.*niger* is industrially important because of its involvement in producing citric acid as well as industrial enzymes, such as amylases, proteases, and lipases. The use of these enzymes is essential because of its importance for transformation to food enzymes. Other properties of this species include pathogens that cause the spoilage of food and production of secondary metabolites, such as aflatoxin, that are toxic. A.*Niger* produce colonies that are composed of white or yellow felt that is covered by dark asexually produced fungal spores. Conidiophores (asexually produced fungal spores) of A. *Niger* usually range from 900-1600 µm in length and contain globular vesicles ranging from 40-60 µm in diameter. Growth takes one to two days on SDB which must be supplemented with antibiotic at a temperature range of 20-28^{0}C.

1.5. STAPHYLOCOCCUS AUREUS*:*

Staphylococci are Gram-positive bacteria, with diameters of 0.5 – 1.5 µm and characterised by individual cocci, which divide in more than one plane to form grape-like clusters. They are non-motile, non-spore forming facultative anaerobes that grow by aerobic respiration or by fermentation. Staphylococcus *aureus* is among the most common hospital acquired pathogens. It is a normal inhabitant of the skin and mucous membranes in the nose of a healthy human. S.*aureus* is infectious to both animals and humans and may only survive on dry skin. It can be spread through contaminated surfaces, through the air and through people. Approximately 30% of the normal healthy population is affected by S.aureus as it asymptomatically colonizes on the skin of human hosts. Though some host colonization can be benign, a puncture or break in the skin can prompt this bacterium to enter a wound and cause infections.

Staphylococcus *aureus* forms a fairly large yellow colony on rich medium. Staphylococci are facultative anaerobes that grow by aerobic respiration or by fermentation that yields principally lactic acid. The bacteria are catalase-positive and oxidase-negative. It can grow at a temperature range of 25 to 40 degrees in Nurtient broth.

1.6. PSEUDOMONAS AERUGINOSA:

Pseudomonas *aeruginosa* is a gram-negative, rod-shaped, asporogenous, and monoflagellated bacterium that has an incredible nutritional versatility. It is a rod about 1-5 µm long and 0.5-1.0 µm wide. It is an obligate respirer, using aerobic respiration (with oxygen) as its optimal metabolism although can also respire anaerobically on nitrate or other alternative electron acceptors. It can catabolize a wide range of organic molecules, including organic compounds such as benzoate. This, then, makes *P.aeruginosa* a very ubiquitous microorganism, for it has been found in environments such as soil, water, humans, animals, plants, sewage, and hospitals.

P. aeruginosa isolates may produce three colony types. Natural isolates from soil or water typically produce a small, rough colony. Clinical samples, in general, yield one or another of two smooth colony types. One type has a fried-egg appearance which is large, smooth, with flat edges and an elevated appearance. Another type, frequently obtained from respiratory and urinary tract secretions, has a mucoid appearance, which is attributed to the production of alginate slime. The smooth and mucoid colonies are presumed to play a role in colonization and virulence. The growth of this organism takes 18-24 hrs at 30-35^{0}C in Nutrient broth.

TABLE 1: Media preparation for the test microorganisms

Medium used for organisms to grow	**Organisms**	**source**
Sabouraud's dextrose agar	Aspergillus *niger*	Procured culture
Modification of Sabouraud's dextrose agar with a few drops of butter	Malassezia *furfur*	Scalp (dandruff)
Nutrient agar/broth	Staphylococcus *aureus*	skin
Nutrient agar/broth	Pseudomonas *aeruginosa*	Procured culture

Above mentioned test microorganisms were isolated using streak plate techniques and also identified by staining techniques, sequenced for confirmation of microorganisms.

2. PROCEDURE:

The leaves of ananus comosus were collected by separating them from the fruit, while the leaves of agava cantala were directly collected from the university campus. The collected leaves were first washed under running tap water for 5 minutes. They were then surface sterilized using tween-20 in 10% v/v concentration for 10 minutes, followed by a 15 minute wash with distilled water(3-4 times) to remove the traces of dirt and chemicals used. A part of these leaves were taken and ground into paste using mortar and pestle (surface sterilized with 70% ethanol). The remaining part was used for fiber extraction.

Extraction of fiber: Fiber from leaves was extracted using chemical retting method. The leaves were soaked in tap water to which 5% solution of NaOH was added. The leaves were left soaked in the solution for about 2 weeks by periodically replacing the solution for every 3-4 days. The leftover solution was removed and the fiber was isolated by thoroughly washing them under tap water followed by distilled water to remove traces of the plant debris. The fibers were then shade dried for 2 days and stored in sterile conditions for further use.

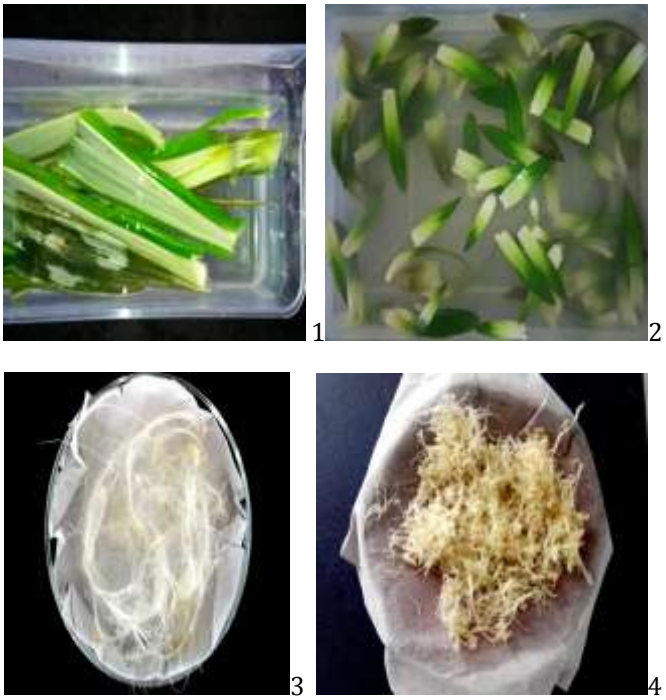


Figure: 1.Agava cantala leaves, 2. pineapple leaves, 3 Extracted Agava cantala fibers, 4.Extracted Pineapple fibers

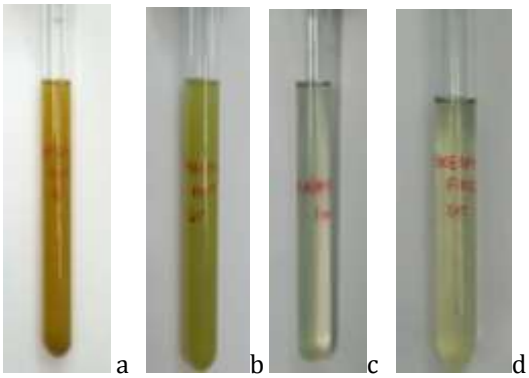
Solvent extractions of the samples were done by using a "Soxhlet extractor". A **solvent** is a substance that dissolves a solute, resulting in a solution. A solvent is usually a liquid but can also be a solid or a gas. The maximum quantity of solute that can dissolve in a specific volume of solvent varies with temperature. The solvent used in the preparation of plant extract in the present study was methanol. Methanol also known as methylalcohol, wood alcohol, wood naphtha or wood spirits, is a chemical with the formula CH_3OH. Because of its low miscibility in water, it is a good general purpose solvent. Its boiling point is 65^{0}C.

Solvent extraction: The plant material is placed inside a thimble made from thick filter paper, which is loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor is placed onto a flask containing the extraction solvent. About 100ml of the solvent was used in case of arils, rind and leaves, and 200ml in case of the dried rind and leaves. The Soxhlet is then equipped with a condenser. The solvent is heated to reflux, at a temperature depending on the boiling point of the solvent involved (60-80^{0}C). The solvent vapour travels up a distillation arm and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapour cools, and drips back down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm solvent. When the Soxhlet chamber is almost full, the chamber is automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. The thimble ensures that the rapid motion of the solvent does not transport any solid material to the still pot. This cycle was allowed to repeat many times, over hours or days.

During each cycle, a portion of the non-volatile compound dissolves in the solvent. After many cycles the desired compound is concentrated in the distillation flask. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled.

After extraction the solvent is removed. The non-soluble portion of the extracted solid remains in the thimble, and is usually discarded.

Soxhlet Extraction process



a b c d

Figure: a. Agava cantala pulp extract, b.pineapple leaves extract, c. Agava cantala fibers extract, d. pineapple fibers extract.

Antimicrobial screening of plant extracts:

Agar well diffusion:

This assay is one of the most convenient, easy and simple means to test the antibiotic sensitivities of microorganisms towards an anti-microbial compound. It involves the diffusion of an anti-microbial compound placed in a well punched into the solidified agar medium containing the inoculated submerged colonies of the test organism. Since the well punched is generally circular, the compound diffuses radically into the inoculated medium. Upon diffusion of the anti-microbial compound, the area of the agar medium present around the well is cleared off the microorganism present, thus showing its anti-microbial effect. This cleared zone is called “the zone of inhibition”. The diameter of the zone of inhibition is measured and recorded, and is dependent on: The sensitivity of the microorganism towards the anti-microbial compound, the effectiveness of the anti-microbial compound on the microorganism.

Procedure: The test organisms were inoculated into respective medium and incubated at certain temperatures for 24hrs. About 20-25ml of the melted agar medium (cooled to a temperature of 40-45^{0}C) was poured into the petri plate and allowed to solidify. About 0.1ml of the culture sample was taken and uniformly spreaded over the solidified agar medium using a sterile glass spreader. A well was punched into the agar, and 120µl of the plant extract was dispensed into the well. The petri dish was labelled and sealed using a parafilm, and incubated overnight at 37^{0}C. This procedure was repeated for all the 4 extracts on each of the four microorganism cultures. Within 24 hours of incubation, a zone of clearance was observed around the plant extract in the agar well plate. The diameter of this inhibition zone was measured in millimetres (mm), and was recorded. The diameters of the inhibition zones of the 4 extracts on each of the four organisms were similarly recorded.

3. RESULTS AND DISCUSSION:

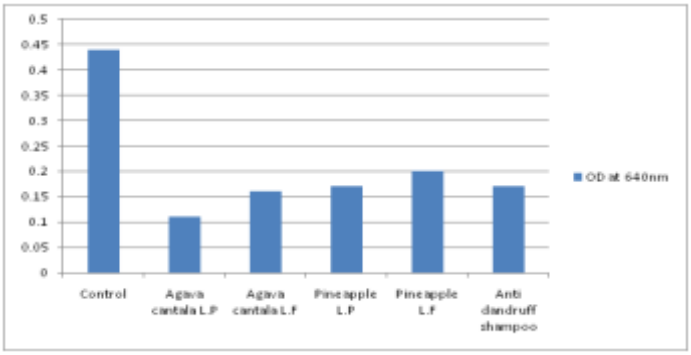
A comparison of the antimicrobial potential of leaf pulp and lead fiber extracts of both the plants was tabulated. The results show that the leaf pulp extracts of both the plants have stronger antimicrobial potential when compared to the fiber extracts. Among both the plants agava leaf pulp extract had a strong antimicrobial effect against all the test organisms. The tabulated results further suggest that the fiber extracts have shown antimicrobial property to some extent.

Dilution method:

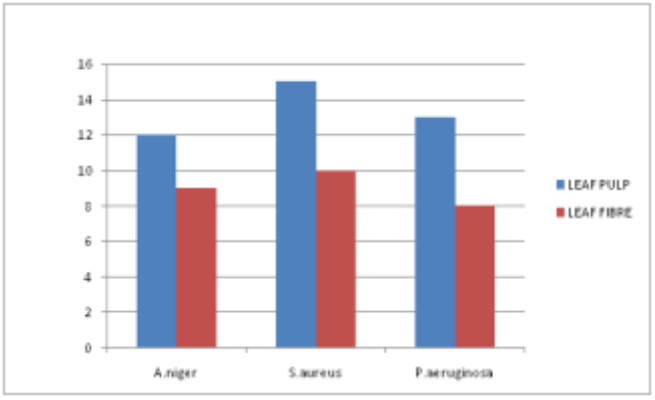
Micro-organisms require sufficient incubation period and temperature for their appropriate growth. These requirements vary from species to species and organism to organism. Malassezia *furfur* need about 4-5 days of incubation period at 37^{0}C. It becomes difficult to test the anti-microbial property of solvents on such micro-organisms using diffusion methods. Hence in the present study the dilution method was adopted to test the antimicrobial sensitivity of Malassezia *furfur* against the solvents by measuring the change in turbidity of the broths and recording the OD vaules using a colorimeter. Turbidity represents microbial growth, while no turbidity represents inhibition of microbes.

About 5 ml of the broth containing Malassezia *furfur* was taken in a test tube and mixed thoroughly using rotary mixer to breakdown the fungal mat. This was used as culture sample in this method. 50 ml of the SD broth was prepared and poured into 5 test tubes 10 ml each with a few drops of butter. The tubes were sterilized in an autoclave for 15min at 15lbs pressure. Upon cooling 1ml of culture sample was inoculated into each test tube and 1 ml of the solvent extract was added. One test tube was kept as control in which no solvent was added. The tubes were kept in incubator for about 3 days. Upon confirmation of sufficient growth, the difference in the turbidity of the test tubes with solvent and without solvent was measured by taking the OD readings using a colorimeter.

Graph1**:** sensitivity of m.*furfur* against plant extracts



Graph-2: comparison of the antimicrobial potential between leaf pulp and leaf fiber of pineapple-well diffusion method



Graph-3: comparison of the antimicrobial potential between leaf pulp and leaf fiber of pineapple against M. *furfur*-dilution method

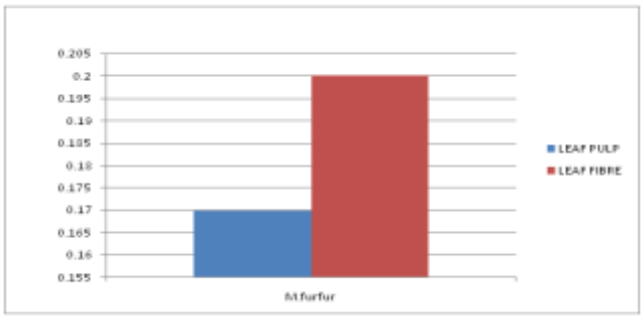


Table-2: Comparison of the antimicrobial potential between leaf pulp and leaf fiber of pineapple

ORGANISM	ZONE OF INHIBITION(mm)					
	LEAF PULP			LEAF FIBER		
	DISC	WELL	DILUTION (OD)	DISC	WELL	DILUTION (OD)
M.*furfur*	-	-	0.17	-	-	0.20
A.*niger*	10	12	-	8	9	-
S.*aureus*	13	15	-	10	10	-
P.*aeruginosa*	13	13	-	10	8	-

4. CONCLUSION

From this study it may be concluded that the leaf pulp and leaf fiber extract of *ananus comosus* and *agava cantala* can be used as potent antimicrobial formulation to treat mild dermatological infections. The leaf extracts of both the plants showed more antimicrobial property when compared to their respective fiber extracts. This study further reveals that fiber which forms from a part of plant skeleton also have antimicrobial property to some extent. The extracts have shown diversity in their antimicrobial property on different microbes. Among the two plants, *agava cantala* extract had more antimicrobial potential on all the tested microbes. Both parts of these plants have almost same compounds. Therefore, both the leaves and leaf fiber can be used in traditional medicine study system for different types of ailments.

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