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NOVEL DRUG DEVELOPMENT FROM VEGETABLE PEELS AGAINST MULTIDRUG RESISTANT PATHOGEN IN TIRUPPUR DISTRICT, TAMILNADU.

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ABSTRACT Peels of various fruits and vegetables are generally considered as waste product and are normally thrown away by us. But different studies conducted on peels revealed the presence of important constituents, which can be used for pharmacological or pharmaceutical purpose.Number of components having activities like anti oxidant, antimicrobial, anti inflammatory, anti proliferative etc. In this study, we planned to conduct a retrospective analysis of the bio active ingredients from vegetable peels of Luffa acutangula, Trichosanthes cucumerina, Lagenaria sincerariapeels, discarded as wastes, from which extracts are prepared & analyzed for the bio active therapeutic agents against MDR pathogens . Development of novel therapeutic bio active potentials from ancient herbal medicines necessitate for the betterment of human welfare becoming a need of an hour. The aqueous peel extract of Lagenaria sinceraria found to contain carbohydrates, saponins and flavonoids. This research pave a path for the budding researchers to focus on novel drug development from agro-waste as raw substance in a precise, cost effective, nutritive and medicinal applications.

KEYWORDS : Luffa acutangula , T. cucumerina, Lagenaria sinceraria, phyto components.

INTRODUCTION

Antibiotic resistance is one of the biggest public health threats worldwide Currently, antibiotic – resistant bacteria kill 700,000 people annually.These data represent the evolving nature of multiple drug resistance which leads to find out a novel drug in the post-anti biotic era in near future. Development of novel therapeutic bio active potentials from ancient herbal medicines necessitate for the betterment of human welfare becoming a need of an hour. Their bio active phytocomponents forms a theme of present research work. In this study, we planned to conduct a retrospective analysis of the bio active ingredients from vegetable peels of *Luffa acutangula, Trichosanthes cucumerina*, *Lagenaria sinceraria* peels, discarded as wastes, from which extracts are prepared. Qualitative and quantitative phyto chemical analysis carried out to find out bio active phyto components. The Multiple drug resistance pathogens from nosocomial origin are collected and screened for their drug resistance pattern. The strains which show MDR are subjected to find out the alternative effective drug formulated from the selected peel extracts. For the present scenario,the bio active components from the peels extract of selected vegetables are more beneficial for the formulation of the novel drug to combat MDR pathogens. This research pave a path for the budding researchers to focus on novel drug development from agro-waste as raw substance in a precise, cost effective, nutritive and medicinal applications.

Lagenaria siceraria (Surai kaayi in tamil; Bottle gourd in english) , *Luffa acutangula* (Peerkankai in Tamil; Ridge gourd in English) and *Trichosanthes cucumerina* (Pudalankai in tamil; Snake gourd in english)(Peels)were collected from in and around Tiruppur district, Tamil Nadu, India. The freshly collected vegetables peels were washed with water and shade dried at under room temperature.The dried materials were powdered in a blender. The powdered material was stored in sterile containers for further use.

MATERIALS AND METHODOLOGY

Collection of vegetable peel sample

Lagenaria siceraria (Surai kaayi in tamil; Bottle gourd in english) , *Luffa acutangula* (Peerkankai in Tamil; Ridge gourd in English) and *Trichosanthes cucumerina* (Pudalankai in tamil; Snake gourd in english)(Peels)were collected from in and around Tiruppur district, Tamil Nadu, India. The freshly collected vegetables peels were washed with water and shade dried at under room temperature.The dried materials were powdered in a blender. The powdered material was stored in sterile containers for further use.

Extraction of sample materials

Aqueous extraction

2.5 gram of dried powders of *Lagenaria siceraria ,Luffa acutangula* and *Trichosanthes cucumerina*(Peels) were suspended in 50ml cold, hot distilled water separately and mixtures were soaked for 24hours.

Solvent extraction

2.5 gram of dried powders of *Lagenaria siceraria , Luffa acutangula* and *Trichosanthes cucumerina* (Peels) suspended in 50ml of solvents (Ethanol, Methanol) separately and the mixtures were soaked for 24 hours.The suspended solids were filtered through Whatmann No.1 filter paper and kept in water bath at 80˚C for 2 hours. The dried crude extracts were stored at 4˚C for further use.

Test pathogens

A ten strains of Clinical wound pathogens Gram negative (*Escherichia coli, Pseudomonas sp., Klebsiella sp., Citrobacter sp.,*)and Gram positive (*Entrococcus* sp., *Staphylococcus* sp.,) were isolated from wound sample collected at Government Hospital Tiruppur, identified by the standard methods.

Antibiotic resistant assay

Kirby-Bauer disc diffusion method was adopted for susceptibility testing. Pure colonies of the isolates were cultured on Mueller-Hinton agar by spread plating. Antibiotic sensitivity discs were then placed on the surface of the culture using a sterile forceps. The plates were incubated at 37°C for 24 hrs. The zones of inhibition were measured using a meter rule.

Antibacterial activity of peels extract

The antimicrobial activity of plant extracts were screened for its antibacterial activity against wound pathogen viz *Escherichia coli., Pseudomonas sp., Staphylococcus* sp.

Agar well diffusion technique (Perez,1990)

The three plant extracts were tested for antibacterial activity by standard agar well-diffusion method against wound pathogen bacteria. The pure cultures of bacterial pathogens were sub cultured on nutrient broth; 20 ml of nutrient agar were poured into petriplates The cultures were swabbed uniformly using sterile cotton swap.Wells of 6mm diameter were made on nutrient agar using gel puncture, respectively and then 100µl of plant extract solution was loaded into the wells. After incubation at 37ºC for 24 hours, the different levels of zone of inhibition were measured.

Phyto chemical Analysis

Qualitative&Quantitative phyto chemical screening was carried out following standard procedures by Harborne,1973, Kokate,1994, Sani *et al*.,2007.

Phyto chemicals analysis using thin chromatography (TLC)(Darabpour *et al.*,2011)

The Hexane + Acetone,Acetone + Ethanol , Ethanol+ Methanol extracts of the most effective parts of *Lagenaria siceraria* was analyzed by TLC; the presence of different constituent types in screened extracts was established by adding 0.5µl of the extracts at 100mg/ml concentration on pre coated silica gel plate. The plate was developed in a chamber saturated with solvent system: Acetone: Methanol: Acetic acid (7.5:08:100µl).

RESULT

Isolation & identification of pathogens

The wound pathogen were isolated from the pus sample. The isolates were identified based on selective plate and biochemical tests. On selective plate, colonies on EMB was found as metallic sheen green colour, on Cetrimide agar was green colour colony observed ;on macconkey agar pink colour colony and pink point was observed; followed by yellow colour colonies on MSA agar.

Antibiotic susceptibility

Susceptibility test was conducted with 10 different antibiotics (Methicillin, Cloxacillin, Ofloxacin, Moxifloxacin, Oxacillin, Penicillin-G, Cefoxitin, Ceftriaxone). The diameter zone of inhibition of antibiotic disc against bacteria species was interpreted as Resistant (R), Sensitive (S) using the zone diameter interpretative chart of the NCCLS (2003) (Table 2, Fig 1)). All the ten strains were resistant to MET (Methicillin). E.coli 1 and *Pseudomonas* sp 1 strain was resistant against all the antibiotics. *Pseudomonas* sp 2 and E.coli 3 resistant against all antibiotic except OF (Ofloxacin) and MO (Moxifloxacin) respectively. Mostly E.coli 1,*Pseudomonas* sp2, *Citrobacter* sp.,*Staphylococcus* sp1, *E.coli* 3 were selected based on their MDR% for further studies.

Antibacterial activity of vegetables peels extract

A total number of 3 vegetable peels extract (Aqueous hot, Aqueous cold, Ethanol and Methanol) from *Lagenaria siceraria, Luffa acutangula and Trichosanthes cucumerina* were screened against 5 bacterial test organisms by the well diffusion method. The results revealed that the methanol extract of *Lagenaria siceraria* was more effective against the test organisms. The *Lagenaria siceraria* peel extract showed superiority in inhibiting *Pseudomonas* sp 2., Staphylococcus sps 1(17mm), while the *Luffa acutangula* peel extract showed superiority in inhibiting *Citrobacter* sp (17mm), E. coli 3 (14mm), *Trichosanthes cucumerina* peel extract of methanol shows the highest zone of inhibition against *E. col*i 3 (20mm), *E. coli* 1(15mm) and for cold aqueous extract showed *Citrobacter* sp.,*Staphylococcus* sp 1(16mm).Other then methanolic extract of *Trichosanthes cucumerina E. co*li1 was resistant to all extracts.

Qualitative analysis of phyto chemicals

The phyto chemical analysis of various extracts using phyto chemistry tests showed that the all peel extracts of *Lagenaria siceraria, Luffa acutangula* and *Trichosanthes cucumerina* contained Alkaloids, Amino acid, Proteins. Other phyto chemicals results were recorded.(TABLE-1)

Table-1 Pytochemicals Result

PARAMETERS	1	2	3
Alkaloids	+	+	+
Flavonoids	+	-	+
Tannin	+	+	-
Steroids	+	-	+
Saponins	+	+	-
Terpenoid	+	-	-
Carbohydrate	-	-	-
Amino acid	+	+	+
Phenol	-	-	-
Triter	+	-	-
Protein	+	+	+
Anthraquiones	+	-	-

1. Lagenaria siceraria
2. Luffa acutangula
3. Trichosanthes Cucumerina

Quantitative analysis of phyto chemicals

Quantitative estimation of Alkaloids, Flavanoids, Protein, Carbohydrate, Tannins, Phenol, moisture and Ash content were evaluated. Quantitative carbohydrate was 56.7 % (*Lagenaria siceraria*), 42.1 % *(Luffa acutangula*) and 37.2% (*Trichosanthes cucumerina*). Protein was 13.8 mg/100g (*Lagenaria siceraria*) 16.8 mg\100g (*Luffa acutangula*) and 10.8 mg\100g (*Trichosanthes cucumerina*). Alkaloids was 2.4% (*Lagenaria siceraria*), 1.6 % (*Luffa acutangula*) and 2.4% *(Trichosanthes cucumerina*). Flavonoids was 2.2% *(Lagenaria siceraria*), 2.33 % (*Luffa acutangula*) and 2.0% (*Trichosanthes cucumerina*) . Ash was 8% (*Lagenaria siceraria*), 7% *(Luffa acutangula*) and 8.5%, *(Trichosanthes cucumerina*).Moisture 7.5 % (*Lagenaria siceraria*), 6.62 % (*Luffa acutangula*) and 7 % (*Trichosanthes cucumerina*). Tannins was 3.00 µgTAE/g (*Lagenaria siceraria*), 6.6 µgTAE/g *(Luffa acutangula*) and 4.6 µgTAE/g (*Trichosanthes cucumerina*). Phenol was 23.2 mgGAE/g(*Lagenaria siceraria*), 20.4 mgGAE/g(*Luffa acutangula*) and22.0mgGAE/g(*Trichosanthes cucumerin*a).

TLC analysis

Hence, the F4th, F6th, F15th, F18th, F21th eluted fractions were selected for purification of active molecules using TLC. shows that, in Bromocresol green produced a yellow-green color on a blue background in four selected fractions to indicate the presence of carboxylic acids in the fractions. Ferric chloride test extracts were treated with 3-4 drops of ferric chloride solution. There was no formation of a bluish-black color, which indicates the absence of phenols. In the Iodine test to observation of brown spot the color on the TLC plate, the selected four fractions were positive. So it indicates organic compounds.

Formulation of nano gel (Mukherjee, 2018)

Chitosan (2.5%) was separately dissolved in 0.5% (v/v) acetic acid solution with continuous stirring and synthesized Nanoparticle of Lagenaria siceraria extract was added. The water-soluble polymers such as PVP(Poly Vinyl Pyrrolidone 10%)and Sodium alginate(0.8%) were solubilised in distilled water. Then solutions at different ratios were mixed by vortexing for 1 h and poured into glass moulds. They were then frozen at −20 °C for 18 h and then thawed at room temperature for 6 h for three consecutive cycles. The hydrogel samples were air dried. They were then soaked in distilled water for removal the soluble parts. Then the gels were then dried.

Figure 1 : Nanogel

CONCLUSION

This research pave a path for the budding researchers to focus on novel drug development from agro-waste as raw substance in a precis&analysed for the bioactive therapeutic agents against the nosocomial pathogens. This first research study could form a foot step for the investigation of novel drug formulations and preparation for the nanogel which could be more efficient get rid of from resistance pathogens.

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