International Journal of Current Trends in Engineering & Technology
ISSN: 2395-3152
Volume: 03, Issue: 06 (NOVEMBER - DECEMBER, 2017)

Comparative Evaluation of Plant Metabolites for Microbicidal Activity and As Bio Preservative

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ABSTRACT: - *Food products entail defence from contamination during their preparation, storing and supply, which give anticipated shelf life. The problem of spoilage of food products can be solved with the development of food preservatives. Food preservatives helps in preservation of food as they can combat constantly against microbes, which are accountable for food spoilage and makes the food hazardous. The current research work is to study the type of microorganisms which typically spoils the food products Escherichia coli(MTCC 40) and Staphylococcus aureus(MTCC 7443) to find the antimicrobial solutions by natural extracts with the help of explants such as Cinnamomum verum, Oreganum vulgare and Withania somnifera. The ethanolic-Methanolic (polar), acetone and Chloroform (non-polar) extracts of the plants were used for examining the antimicrobial activity. On the basis of observation of results, the extracts of three different solvents were combined with the Iron metal ion , Fe++ [3].The best results of zone of inhibitions were optimized for the development of bio preservatives.*

KEY WORDS: Antimicrobial properties, ethanolic and methanolic extracts, polar and non-polar, zone of inhibition, metal ions.

INTRODUCTION

To study the effect of plant metabolite complex as an antimicrobial agent and bio preservative, the selection of plant plays a vital role. As the plant metabolites should greatly show a higher degree of resistance towards the food spoiling microorganisms and then are later characterized to develop as a food bio preservative by coupling to the Iron metal ion. For such reason, the plant breed selection is a time-consuming process. After all, the plant should be showing minimum toxic effect ad evoked response against food spoilage [6]. Also, the main aim is to culminate the plant metabolite to a metal ion to develop a biofilm, the film should be cheaper and even after wrapping the food with this should show nullified reactivity to food and act as a good preservation material. Therefore, the direction of project work was taken such that identification of plant, spoiling the food, isolation of plant extracts, culturing the organism in the plates, study the effect of plant extract on to the plated organisms and if the zone of inhibition is found it is then carried out to characterize the plant metabolite. *Withania somnifera* (Ashwagandha), *Oreganum vulgare* (Oregano) and *Cinnamomum verum* (Cinnamon) have been selected for testing of antimicrobial activity.

MATERIALS AND METHODS

Plant selection: The roots of Ashwagandha were purchased online; leaves of oregano and bark of cinnamon were purchased from departmental stores of Lovely Professional University, Phagwara, and Punjab.

Drying of plant raw materials: The barks of cinnamon, leaves of oregano and the roots of ashwagandha were washed to get rid of the dirt particles. The dried plant parts were incubated for one day at 37 degrees centigrade. After they were completely dried the plant parts were ground to a fine powder individually in a mixer. The obtained powder was collected and run for soxhlet extraction.

Acetone, chloroform, ethanol-methanol plant extraction: The plant raw materials of Cinnamon were washed with double distilled water and carefully dried in shade. After they are dried completely, grind 20 gms of cinnamon with the help of mortar and pestle to make into small bits and pieces of aqueous using 150 ml of double distilled water and methanolic extracts using 150 ml of methanol respectively and pour them in beakers and keep in shaking incubator for five to eight hours after that they are sealed transferred into refrigerator for storage. 20gms of Cinnamon bark was finely ground and placed in a whatman filter paper. Ethanol was taken as a polar solvent, acetone and chloroform were taken as non-polar solvents and the extracts were obtained using soxhlet apparatus. The extracts were stored in clean and dry place without contact with the direct sunlight. The solvents used were acetone-slightly polar, chloroform highly polar for soxhlet extraction and ethanol-methanol-distilled water in proportions of 1:3:1 and they were carefully wrapped with the help of aluminum foil and kept in dark for one week, after that the wrap was removed and dissolved in tris-Hcl solution(1mg/ml). The obtained soxhlet solutions of cinnamon, oregano and ashwagandha were obtained in pure extract form by extraction.

Bacteria tested for antimicrobial activity:
Microbiological techniques utilized for the testing of antimicrobial activity of Ashwagandha, oregano and cinnamon by agar well diffusion. Streptomycin, Amoxicillin and potassium clavulnate were used as controls.

Minimum Inhibitory Concentration:
Minimum inhibitory concentration has been observed by agar well diffusion method. The plates containing agar medium were spread with either 1 ml of the bacterial inoculum. Wells were made using micro tips and also gel puncture was used for making agar well diffusion wells. The plates are kept in incubator set at 37°C for 24 h and the diameter of result inhibition zone was calculated [8]. Five different concentrations of the plant metabolite extracts were prepared. Stock concentration of every extract was read as 100mg/ml. From the stock extract 1ml was collected and diluted with 99ml of distilled water. The process was repeated for four times for obtaining three different concentrations.

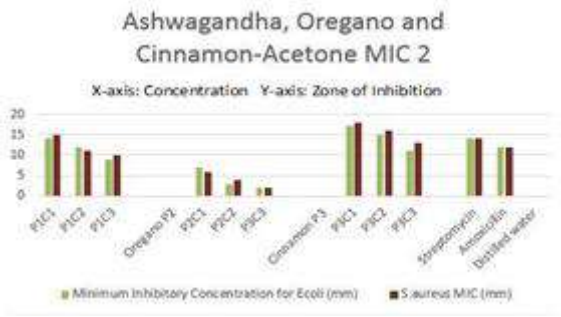
Figure 1: Control consisting zone of inhibition for antibiotics streptomycin and amoxicillin & potassium clavulanate and distilled water, plant acetone extracts and chloroform extracts on *E.coli.*

Activity of Iron metal ion: After the process of conjugating the plant extracts with iron metal ion was done to test for the biocompatibility with well-known metal iron in the form of its salt solution in polar and nonpolar solvent form. For this process the acetone, methanolic and chloroform plant extracts of Ashwagandha, Oregano and Cinnamon were conjugated with metal-ion to observe the biocompatibility.

Figure 2: Zone of Inhibition of Plant acetone extracts + Metal ion for E.coli, chloroform extracts + Metal ion iron for E.coli, and control streptomycin, amoxicillin and metal ion alone, Ashwagandha, Oregano and cinnamon combined together with metal ion iron for E.coli, control for Staphylococcus aureus streptomycin, amoxicillin & potassium clavulnate and metal ion alone, plant chloroform extracts + metal ion, Ashwagandha, Oregano and cinnamon conjugated with metal ion iron for Staphylococcus aureus.

Different concentrations 100 microlitres namely 10% concentration of metal ion C1(90microlitre of Plant extract+10microlitre of Iron)-1mg/ml, 20% concentration of metal ion C2(80 microlitre of plant extract+20 microlitre of metal ion)- 0.8 mg/ml and 30% concentration of metal ion C3(70 microlitre of plant extract+30 microlitre of metal ion)- 0.7 mg/ml. Compared with the antibiotics Amoxicillin, Streptomycin and Iron metal ion alone [9].

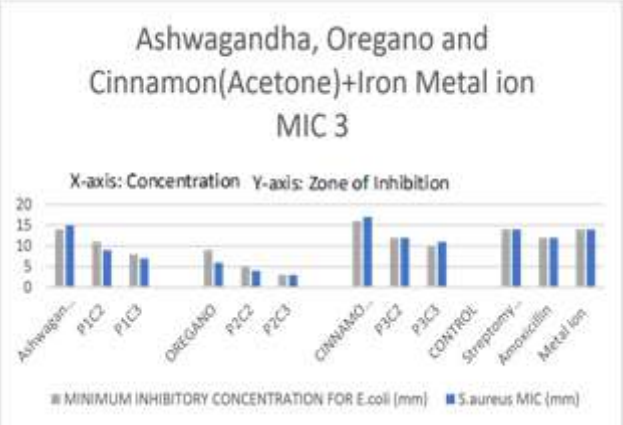
RESULTS



Graph 1: Ashwagandha, Oregano, Cinnamon-Acetone MIC 2

Table 1: Minimum Inhibitory Concentration of plant Acetone extracts alone at three different concentrations C1, C2 and C3.

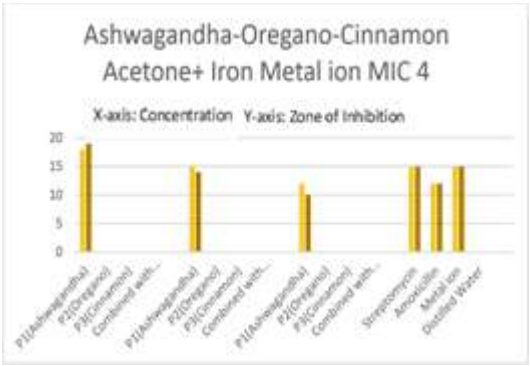
Plant extracts	MIC	*S. aureus*
Acetone	*E.coli*	
Ashwagandha P1		
P1C1	14	15
P1C2	12	11
P1C3	9	10
Oregano P2		
P2C1	7	6
P2C2	3	4
P2C3	2	2
Cinnamon P3		
P3C1	17	18
P3C2	15	16
P3C3	11	13
Streptomycin	14	14
Amoxicillin	12	
Distilled water	0	



Graph 2: Minimum Inhibitory Concentration of plant Acetone extracts conjugated with Iron metal ion at three different concentrations C1, C2 and C3.

Plant extracts Acetone +Iron metal ion	MIC(mm)	
	E.coli	***S. aureus***
Ashwagandha P1		
P1C1	14	15
P1C2	11	9
P1C3	8	7
Oregano P2		
P2C1	9	6
P2C2	5	4
P2C3	3	3
Cinnamon P3		
P3C1	16	17
P3C2	12	12
P3C3	10	11
Streptomycin	14	14
Amoxicillin	12	12
Iron metal -ion	14	14

Table 2: Minimum Inhibitory Concentration of plant Acetone extracts conjugated with Iron metal ion at three different concentrations C1, C2 and C3.



Graph 3: Ashwagandha-Oregano-Cinnamon Acetone+ Iron Metal ion MIC 4

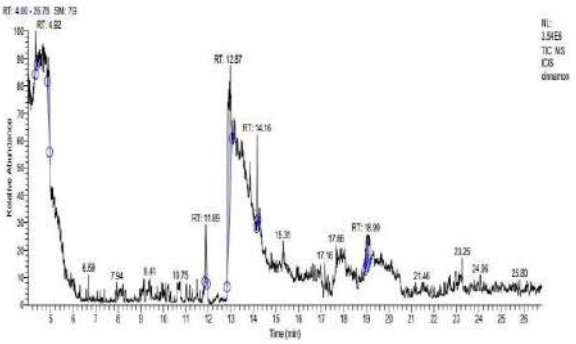
Plant extract (Acetone)	MIC(mm)	
	E-coil	
P1(Ashwagandha)	10	19
P2(Oregano)		
P3(Cinnamon)		
Combined with Metal ion at C1		
P1(Ashwagandha)	15	14
P2(Oregano)		
P3(Cinnamon)		
Combined with Metal ion at C2		
P1(Ashwagandha)	12	10
P2(Oregano)		
P3(Cinnamon)		
Combined with Metal ion at C3		
Streptomycin	15	15
Amoxicillin	12	12
Iron Metal ion	15	15
Distilled Water	0	0

Table 3: Minimum Inhibitory Concentration combined plant acetone extracts conjugated with iron metal ion at three different concentrations C1, C2 and C3.

The acetone extract of Cinnamon was evaluated by GCMS to Central Instrumentation Laboratory of

Punjab University for identification of effective plant metabolite present in it.

Gas Chromatography Mass Spectrometry (GCMS):



From the GCMS results obtained, the metabolites Lycoxanthin Phenol, 2-methoxy-6-nonamethyl hexatriacontanonaenyl showed its retention time at 11.8 highest relative abundance peaks and Guanosine, 2'-deoxy- Methanocyclopenta cyclopropa cyclodecen-11-one, Fenretinide at 12.87, trans(2-Chlorovinyl) dimethylethoxysilane 14.16 Phthalic acid, butylundecyl ester. Lutein at 19.05 HCyclopenta[a]cyclopropa[f]cycloundecene 2,4,7,7a,10,11hexol,1,1a,2,3,4,4a,5,6,7,10,11,11adodeca hydro-1,1,3,6,9-pentamethyl-,2,4,7,10,11 pentaacetate Milbemycinb-19.5RT,13-chloro-5-Odemethyl-28-deoxy-6,28-epoxy-25-(1-methylpropyl)-, [6R,13S,25R(S)] at Retention time 19.5 showed maximum level of relative abundance which might be responsible for the antimicrobial activity of Cinnamon.

CONCLUSION

From the results obtained it was clearly observed that Acetone extracts of Cinnamon, maximum inhibitory concentration followed by methanol and finally chloroform. It was concluded that Acetone cinnamon and methanol metabolite extracts when conjugated with the iron metal ion have maximum antimicrobial activity towards food spoiling pathogens when compared to antibiotics Streptomycin and Amoxicillin and Potassium clavulnate. The selected plants acetone extracts with essential metal ions at a pre-requisite concentration shows a significant anti-microbial efficacy compared to plant extracts used alone. The outcome of this research work can be further applied in preparing biofilm with the plant based extracts and can be used to as bio-packaging agent which significantly increases the shelf life of the food products.

Acknowledgement:

We are thankful to School of Bioengineering and Biosciences project laboratory of Lovely Professional University for encouragement and support.

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