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ANTIMICROBIAL ACTIVITY OF PHYTOCHEMICALS EXTRACTED FROM *ABELMOSCHUS ESCULENTUS* AGAINST CHOSEN PATHOGENS

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ABSTRACT

Plants have been evaluated on various aspects of its property from ancient times. The various chemical constituents with greatest therapeutic property are identified. The effect of microorganism can be tackled with an effective immune system. Therefore the stimulation of defense mechanism against bacterial, fungal or viral infections has been proven in effective manner. To Control and control of various disease requires effective immunomodulators as important remedial method with consisting phytochemicals.

Keywords: *phytochemicals, antimicrobial activity and pathogens.*

INTRODUCTION

Phytochemicals defined in the strictest sense, as chemicals produced by those plants. However, the term is generally used to describe chemicals from plants that may enhance health status of organisms, but are not necessary nutrients (Saurabh Srivastava *et al*., 2011). There is an evidence to support the health benefits of the diet in the form of fruits, vegetable, legumes, whole grains and nuts (Mojab *et al*., 2003). Because plant based foods products are crude of bioactive compounds, information on the potential health of individual phytochemical is linked to information on the health effects of foods that contains various phytochemicals (Manjula *et al*.,2009).

Medicinal Plants are rich in secondary metabolites and are potential source for drugs and essential oils. Steroids, alkaloids, glycosides, insecticide, additives and related active metabolites found in plants are of great value in the drug and pharmaceutical industry (Khatune *et al*.,2005). *Abelmoschus esculentus* L. is broadly taken as a raw vegetable in both temperate and tropical countries. Although the seed pods are most often used, the mature seed is known to have superior nutritional quality. Plant sample has been increasing interest in discovering new natural antimicrobials (Sagdic *et al.,* 2003). Plant

products with antimicrobial properties notably have obtained emphasis for a possible application in food production in order to prevent bacteria and fungal growth (Lanciotti *et al*., 2004). Antimicrobial activity of several plant metabolites have been extensively documented (Khatune *et al.,* 2005).The report was made by Rubatzky and Yamaguchi in the year 1997 representing the seed of *Abelmoschus esculentus*is one of the rich source of protein and as well as oil. Ndangui *et al*.(2010) stated *A. esculentus* seeds could be considered as good sources of protein and minerals and high unsaponifiable content. The physical characteristics of *A. esculentus*are compared with jute fiber (Sen et al. 1987). *A. esculentus*fiber may be white in colour, light cream or yellow in color. This was identified for the good mechanical strength (Fathima and Balasubramanian, 2006).

MATERIALS AND METHODS

A. esculentus was collected from organic farm located at Thiruvallur with Latitude and Longitude of 13.1231° N, 79.9120° E. The collected samples were authenticated by Botanist using standard keys and descriptions and confirms with the help of herbarium. The immatured *A. esculentus* fruit was washed with normal tap water and then it was further shade dried. Then, dried plant was grinded. After that, 20 gm of crushed matetial was mixed in 200 mL solvent of methanol. To perform the extraction, Soxhlet extractor process was followed.

The pathogens *Staphylococcus aureus, Streptococcus fecalis, Salmonella typhi, Escherichia coli, Pseudomonas aerogenosa and Bacillus subtilis* were collected from Arvind eye hospital lab, Tirunelveli, Tamilnadu, India. Those culture was maintained in the basal media. The disc diffusion method on the agar was followed for determining the antimicrobial potential by following the procedure of Garg and Jain, 1998. For the preparation of disc Whatman No.1 filter paper of 6-mm diameter used. It was placed in dry Petri plates, were autoclaved. The test extracts in measured quantities were dissolved in minimum amount of acetone. The sterile paper discs were loaded with the extracts of *A. esculentus*prepared using various solvents. The amount of extracts loaded in each disc was in the concentration viz., 25µg/ml, 50µg/ml, 75µg/ml and 100µg/ml. Similarly discs were prepared for standard antibiotic tetracycline (w/v) and were impregnated in the filter paper discs in different concentrations (25 and 50 µg/ml).

MIC and MBC test: Minimum Inhibitory Concentration (MIC) of the extracts was determined from the culture plates that had the lowest concentrations and prevented the growth of bacterial strain. Minimum Bactericidal Concentration (MBC) was determined by using the method of Yoshiki *et al.,* (1998). The tender fruit extracts of *A. esculentus* were

diluted to obtain concentration ranging from 10 µg -100 µg /ml. The test tube containing 3ml of Muller Hinton broth and 0.1 ml bacterial suspensions and 0.1 ml plant extract were incubated at 37^{o}C for 24h. Bacterial turbidity was measured at 650 nm to determine bacterial inhibition. Streptomycin at 20 and 40µg /ml was used as a reference for determination of minimum inhibitory and bactericidal concentrations respectively.

RESULTS AND DISCUSSION

The various solvent extracts Hexane, Butanol, ethanol, Chloroform and Aqueous were sticky in nature. It was diluted maintained as stock solution for further use. The Rf value for the various extracts were discussed in the table 1. The maximum the number of the compounds were separated in ethanol extracts and it was eluted 5 different compounds. Hexane extract and butanol extract eluted 4 compounds. Chloroform and aqueous extract were eluted only 3 compounds. The Rf value for each compound was listed in table 1. for the respective extracts.

Antibacterial activity of fruit extracts of *A. esculentus* were evaluated by measuring the zone of inhibition against human bacterial pathogen and the results were presented in Figure 1. The hexane extract was showing the maximum zone of inhibition 17 mm in diameter against*P. aeruginosa* using 100 mg/*ml concentration.* Against with *E. Coli and S. typhi* it was showing 16 mm in diameter using 100 mg/ml concentration. Against *S. aureus and B. subtilis* it was showing 14 mm in diameter at same concentration. Against *S. faecalis* it was showing 13 mm in diameter. That was least inhibition.

In butanol extract against *B. subtilis* it was showing 18 mm in diameter. Ethanol extract shows maximum (17 mm) against *E. coli*. Chloroform extract shows 17 mm in diameter against *P. aeruginosa and S. faecalis*. The aqueous extract shows 17 mm in diameter against *S. aureus* sp. It was found that butanol extract was showing greater effect on all pathogens compared to all other extracts. De et al. (2011) reported that the gold nanoparticles derived from the pulp of okra exhibited significant microbicidal effectiveness against Bacillus cereus, Bacillus subtilis, E. coli, P. aeruginosa, and M. luteus. Moreover, it has been shown that okra pod fractions high in carbohydrates have anti-Hippobacter pylori properties (Lengsfeld et al., 2004).

The Minimum inhibitory concentration of *A. esculentus* (fruit) hexane extract represents the result as 0.7, 0.6, 0.3, 0.4, 0.6 and 0.5 respectively for the organisms *S. aureus, S. faecalis, B. subtilis, E.coli, S. typhi* and *P. aeruginosa*. The best result was found at against *B. subtilis* with minimal concentration to control. The butanol extracts showing the best result by order 0.3, 0.4, 0.5, 0.5, 0.6, 0.8 for *S. typhi, P. aeruginosa, S. faecalis, E.*

coli, B. subtilis and *S. aureus* respectively. The ethanolic extract shows inhibition at least concentration by order 0.2, 0.3, 0.3, 0.3, 0.4, 0.4 respectively for *S. typhi, P.aeruginosa, S.aureus, B.subtilis, S.feacalis and E.coli*. The chloroform extract shows inhibition at least concentration as 0.3, 0.6, 0.6, 0.6, 0.7, 0.9 for *P.aeruginosa, S.typhi, E.coli, B.subtilis, S.feacalis, S.aureus* respectively. The aqueous extract shows inhibition of 0.2, 0.3, 0.3, 0.4, 0.4, and 0.5 respectively for *P.aeruginosa, S.typhi, B.subtilis, S.aureus, E.coli, and S. feacalis* (Table 2).

The control tetracycline shows the result of inhibition at least concentration of 0.1, 0.1, 0.2, 0.2, 0.3 and 0.3 respectively for *S.typhi, P.aeruginosa, E.coli, B.subtilis, S.feacalis, and S.aureus* respectively. The extract found as best for the pathogen *P.aeruginosa,* was aqueous extract. It was showing 0.2 which is near by the activity of tetracycline (0.1). For the pathogen *S. typhi*, the ethanol extract was found best with inhibitory concentration. For *B. subtilis*, aqueous, ethanol and hexane extract was found best with inhibitory activity at least concentration. For *E. coli*, there was not showing must effective nearby tetracycline inhibitory concentration. For S. aureus, ethanol extract was found at the best least concentration as same like tetracycline. For *S. faecalis*, ethanolic extract was found best with least inhibitory concentration (Table 4.2). The Minimal bactericidal concentration is represented in Figure 2 which is similar to the Minimal inhibitory concentration of the extract.

The phytochemical analysis results shows the qualitative analysis of steroids, reducing sugar, sugars, alkaloids, phenolic compounds, flavonoids, tannins, saponin, aminoacids, glycosides and terpenoids (Table 3.). Among all the tested phytochemcials in test sample the composition arranged in the order of terpenoids, saponins, steroids, sugar, tannin, phenolic compound, aminoacids, alkaloids, flavanoids and glycosides (Table 3). Plant based foods products are crude of bioactive compounds, information on the potential health of individual phytochemical is linked to information on the health effects of foods that contains various phytochemicals (Manjula *et al*., 2009). However, plant and their parts and the pattern of administration vary from person to person. Thus there is an enormous scope for tribal medicine based on plant products which are yet to be studied, analyzed and documented scientifically (Warrier *et al*., 1996 and Ramasubbu and Chandra Prabha, 2009).

SUMMARY AND CONCLUSION

A. esculentus was collected from organic farm located at Thiruvallur District. It was allowed to be dried at room temperature followed by grinding. The physical

characterization were performed to check the quality of the fruit for laboratory analysis.The sample was regular in morphological structure with green and fresh. The length of the fruit was identified as 15.3 cm. The various solvent extracts Hexane, Butanol, ethanol, Chloroform and Aqueous were prepared and evaluated for antimicrobial property against pathogens *Staphylococcus aureus, Streptococcus fecalis, Salmonella typhi, Escherichia coli, Pseudomonas aerogenosa and Bacillus subtilis*. It was found that the maximum zone of inhibition was identified with chloroform extract of maximum 18 mm zone of inhibition against*E. coli.*

The Phytochemical constituents were evaluated for the qualitative analysis of the extract. The Minimum inhibitory concentration of *A. esculentus* (fruit) hexane extract represents the result as 0.7, 0.6, 0.3, 0.4, 0.6 and 0.5 respectively for the organisms *S. aureus, S. faecalis, B. subtilis, E.coli, S. typhi* and *P. aeruginosa*. The best result was found at against *B. subtilis* with minimal concentration to control. The butanol extracts showing the best result by order 0.3, 0.4, 0.5, 0.5, 0.6, 0.8 for *S. typhi, P. aeruginosa, S. faecalis, E. coli, B. subtilis* and *S. aureus* respectively.The extract found as best for the pathogen *P.aeruginosa,* was aqueous extract.

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Table 1. Crude extract analysis of various solvents.

Solvent system	**No. of spots / Rf value**	***A. esculentus*fruit extracts**				
		Hexane	**Butanol**	**Ethanol**	**Chloroform**	**Aqueous**
Toluene – ethyl formate – formic acid (5:4:1)	**No. of spots**	4	4	5	3	3
	Rf values in cm					
	Compound 1	0.13	0.05	0.06	0.17	0.08
	Compound 2	0.15	0.09	0.11	0.23	0.18
	Compound 3	0.27	0.11	0.18	0.44	0.37
	Compound 4	0.32	0.28	0.21		
	Compound 5			0.28		

Table 2. Minimum inhibitory concentration *A. esculentus* (fruit) extracts against human pathogenic bacteria organisms.

Solvent	**Minimum inhibitory Concentrations (mg/ml)**					
	S.aureus	*S.feacalis*	*B.subtilis*	*E.coli*	*S.typhi*	*P.aeruginosa*
Hexane	0.7	0.6	0.3	0.4	0.6	0.5
Butanol	0.8	0.5	0.6	0.5	0.3	0.4
Ethanol	0.3	0.4	0.3	0.4	0.2	0.3
Chloroform	0.9	0.7	0.6	0.6	0.6	0.3
Aqueous	0.4	0.5	0.3	0.4	0.3	0.2
Tetracycline	0.3	0.3	0.2	0.2	0.1	0.1

Table 3. Phytochemical analysis of various extract

S. No.	**Phyto-constituents**	***A. esculentus* fruit extracts**				
		Hexane	Butanol	Ethanol	Chloroform	Aqueous
1.	Sterols	++	-	+	+	+
2.	Reducing Sugar	–	–	–	-	-
3.	Sugar	++	-	-	+	-

4.	Alkaloids	-	-	-	+	+
5.	Phenol	++	-	-	-	-
6.	Flavanoids	-	-	++	-	-
7.	Tannins	++	-	-	+	+
8.	Saponins	+	-	++	+	+
9.	Amino acid	+	++	-	+	-
10.	Glycosides	-	-	+	-	+
11.	Terpenoids	+	++	-	+	+

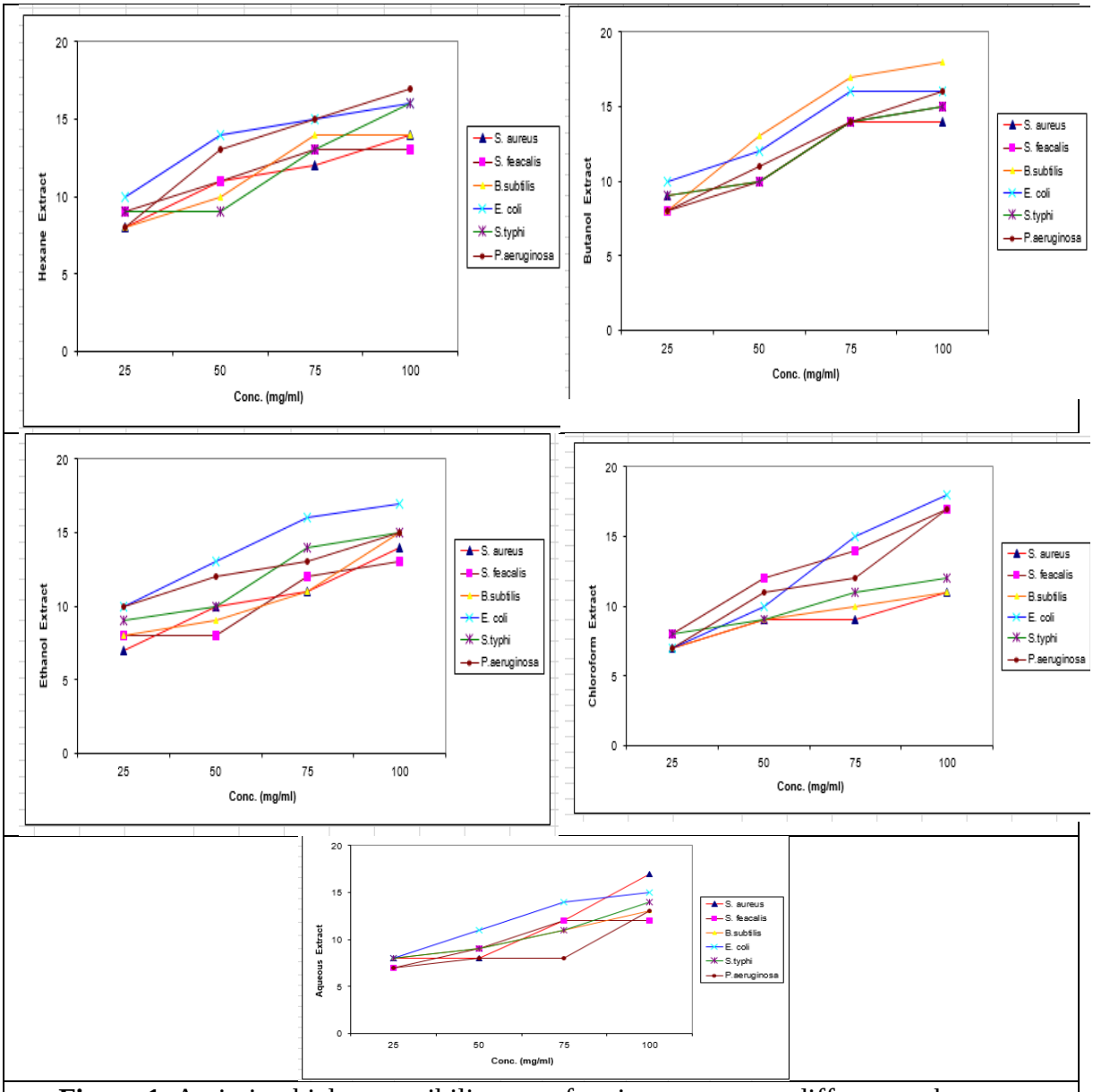

Figure 1. Antimicrobial susceptibility test of various extract on different pathogens.

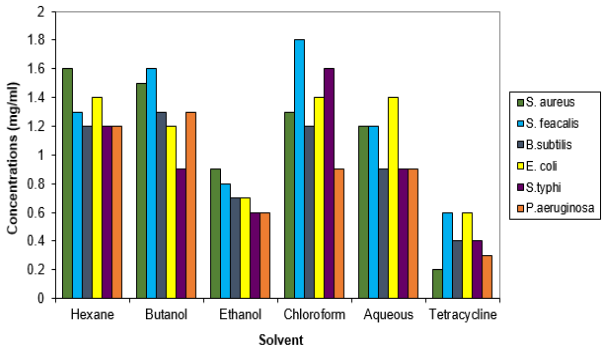

Figure 2. Minimum bactericidal concentration of *A. esculentus* (fruit) extracts against human pathogenic bacteria organisms.