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STUDY OF ANTIOXIDENT AND ANTIMICROBIAL ACTIVITY OF *PHALARIS CANARIENSIS* SEED IN DIFFERENT EXTRACTS

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ABSTRACT

Phalaris canariensis seed extracts are used to assess their antimicrobial and antioxidant activity. Extracts are made through polarity decreasing order od solvents where aqueous, ethanol, ethyl acetate, chloroform, cyclohexane and pet ether were used. After comparing with the standard Ciprofloxacin we found that Aqueous extracts of canary seeds gave the highest activity against all the experimented microbes that was 22, 16, 12 mm of ZI (zone of inhibition) against *E.coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus.* After performing the DPPH assay seed extracts of canary has a good apability of inhibiting free radicals.

KEYWORDS: *Phalaris canariensis,* Antioxident, Antimicrobial Activity, *E.coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, DPPH.

INTRODUCTION

Before the modern medicines existed, the primitive people were dependent on plant parts for treatment of diseases. These health care practices were used for a long period of time and is termed as traditional medicine. As time went by, the remedies used were modified by indigenous cultures.[1,9] Significant hard work has been given for the discovery and growth of natural drug from plants. (Stabilizing, of, Adnata, & Fractions, n.d.) Parts of plants like roots, barks and leaves are used to treat diseases form long time back.[2,3] Different plants are being used as the most common form of medicine for several aboriginal people.[10] The use of plant extracts as medicines is a better alternative to allopathic because of the less to no side effect.

They are also more economical.[11] The reason behind emergence of traditional medicine even with the existence of allopathic medicines is because the modern medicines are being resistant by the numerous pathogens at a very elevated rate.

The canary seed or *Phalaris canariensis* is a member of a family of grasses called Graminaceae. It has been known to be used in folk medicine in the form of tea as a co-adjuvant in the management of hypertension, diabetes mellitus, and hypercholesterolemia.[5,6] Canary seeds have been also been used as a treatment for kidney, pancreas, bladder diseases, and also obesity. Their application in human health is growing due to their antihypertensive property, inhibition of urinary tract infections, arteriosclerosis, AIDS, gout, rheumatism, edema, gastritis and stomach ulcer. The seeds also provide muscle tone and prevent cardiovascular disorders.[8] The grain is grown in various parts of the temperate climates; its production is at present concentrated in the southwestern territory of Canada and, on a much smaller scale, in Argentina, Hungary, North Africa, the Middle East, the United States and Australia.[3] Canary seeds hold nearly 60% starch, 20% protein, 7% total dietary fiber, and 8% crude fat. Canary seed starch (CSS) contains small granules with comparatively low amylose content which ranges from 16.2–19.5%.[4] The major use of canary seed is bird food, alone or combined with extra grains like millet, sunflower seeds, and flaxseeds. The main carotenoid compounds that were identified in canary seed are lutein, zeaxanthin, and β-carotene. Canary seeds hold up to 21% of proteins. The seeds are abundant in cysteine, tryptophan, and phenylalanine which constitutes up to 55%, oleic content is 29%, palmitic content is 11%, and linolenic acids is 2.5%.[1] The sterol along with triterpene alcohol esters of caffeic acid are the chief effective antioxidant components in the crude oil of canary seeds. The most important carotenoid compounds that are identified in canary seed are lutein, zeaxanthin, and β-carotene.[3] Canary seed is also a rich source of vitamins and minerals. Furthermore, canary seed contains abundant phenolics and carotenoids.[7] To further investigate the properties of the seed, our study focuses on its antimicrobial and antioxidant properties which can contribute to the modern medical sciences.

MATERIALS AND METHODS

Collection of the seeds: The plant *Phalaris canariensis* seeds were collected from the local area of Dhaka, Bangladesh in the month of November, 2017. The seeds were freed from materials like dust, dirt, polen. Then the plant was identified by Bushra Khan, Principal

Scientific Officer, Bangladesh National Herbarium, Mirpur, Dhaka and a voucher specimen has been deposited (DACB:42,432) for further reference.

Extraction of seed material: The seeds were dried under sun for a few days and finally oven dried to remove all the moisture content. Then the seeds were crushed to coarse consistency. The coarse grains were extracted in a decreasing polarity order. The coarse plant material (900g) was taken and soaked with 1500 ml of methanol for 3 consecutive days at 25ºC. The extract was filtered and the filtrate was kept for further extraction. In the same manner the filtrate was soaked in different solvents by polarity decreasing order.
Aqueous > Ethanol > Ethyl Acetate > Chloroform > Cyclohexane > Pet ether

For every case, the extract was preserved and solvent evaporation was done by using rotary evaporator. Finally, all the extracts of *Phalaris canariensis* was kept under laminar airflow for protecting it from any type of contamination.

Drugs and chemicals: Ciprofloxacine was assorted from Eskayef Bangladesh, DPPH was from Sun Impex Chemicals and other reagents were of analytical grade.

Collection of Microbes: Bateria were collected from the School of Mathematics and Natural Sciences, BRAC University (*E.Coli* and *Pseudomonas Aeruginosa)* and; from the dept. of Microbiology, Jahangirnagar University (*Staphyloccus Aureus)*. All bacterial cultures were collected in Tryptone Medium and freeze-dried in glycerol stock medium for subsequent usage.

Antimicrobial Assay: Antimicrobial activity of *canary seed extract* was performed using by disc diffusion assay technique, against three bacteria (*E.Coli*, *Pseudomonas Aeruginosa* and *Staphyloccus Aureus)*. A total of 9 Discs, each of 9mm diameter were taken and coated with the extract, then placed across the bacterial cultures with proper spacing among each discs; with 3 discs for each colony. The dishes were left to incubate overnight at 37.5^0C. Later, Zone of Inhibitions (Zi) were recorded.

Determination of anti-oxidizing capacity (DPPH assay): DPPH assay was used to determine the anti- oxidant activitiy of canary seeds. It focuses on the presence of stable 1, 1-diphenyl 2-picrylhyorazyl (DPPH) free radical as previously described by Brand-Williams *et al* with minor modifications. 1 ml of canary seed extract was thoroughly mixed with 1ml of 0.1mM DPPH solution in methanol at different range of concentrations (15, 20 and 25

mg/ml). A reference standard Corresponding blank sample was prepared using L-Ascorbic acid (1-100 µg/ml). Next, a mixture of 1 ml DPPH reagent and 1 ml ethanol was used as control. The reaction was carried out to obtain triplicate readings and the absorbance decrease was measured at 517nm, after 30 minutes in dark using UV-Vis spectrophotometer, according to Sahu *et al, 2013*. The percentage inhibition was calculated using the formula:

Inhibition % = Ac-As/Ac×100

Where Ac is the absorbance of the control As is the absorbance of the sample.

Statistical Analysis

Antimicrobial assays were performed in triplicate under strict aseptic conditions to ensure consistency of all findings. For each extract triplicate data was taken and the final data was taken by the triplicate data's mean ± SD (Standard Deviation), which was analyzed by Microsoft excel.

RESULTS AND DISCUSSION

Antimicrobial activity: In all the extracts, it is seen that the zone of inhibition for E. Coli is greater compared to both Pseudomonas aeruginosa and Staphyococcus aureus. This represents that extract of *Phalaris canariensis* dissolved in different solvent is able to work better against E.Coli than against Pseudomonas aeruginosa and Staphyococcus aureus. It can also be concluded that the aqueous extract works best among all the other extracts that has been tested in this paper. Table 1.1 shows the name of the extract, name of the organism along with the zone of inhibition with average and standard deviation. Triplicate data was taken in order to increase precision for the results.

Table 1: The table shows the zone of inhibition of different organism against different solvent extract of *Phalaris canariensis.*

Name of extract	Name of the organism	Zone of inhibition/mm			Average	Standard Deviation
aqueous	*E.Coli*	21	24	22	22.3333333	1.527525232
	Pseudomonas aeruginosa	18	15	16	16.3333333	1.527525232
	Staphylococcus aureus	12	13	13	12.6666667	0.577350269
ethanol	*E.Coli*	20	22	21	21	1
	Pseudomonas aeruginosa	12	13	12	12.3333333	0.577350269
	Staphylococcus aureus	10	11	10	10.3333333	0.577350269
ethyl actate	*E.Coli*	19	22	20	20.3333333	1.527525232
	Pseudomonas aeruginosa	12	12	10	11.3333333	1.154700538
	Staphylococcus aureus	14	11	11	12	1.732050808
chloroform	*E.Coli*	19	19	21	19.6666667	1.154700538
	Pseudomonas aeruginosa	12	11	10	11	1
	Staphylococcus aureus	12	13	12	12.3333333	0.577350269
cyclohexane	*E.Coli*	19	18	16	17.6666667	1.527525232
	Pseudomonas aeruginosa	12	11	11	11.3333333	0.577350269
	Staphylococcus aureus	12	10	10	10.6666667	1.154700538
Pet ether	*E.Coli*	14	14	12	13.3333333	1.154700538
	Pseudomonas aeruginosa	11	11	12	11.3333333	0.577350269
	Staphylococcus aureus	12	10	11	11	1
standard (ciprofloxacin)	*E.Coli*	34	33	31	32.6666667	1.527525232
	Pseudomonas aeruginosa	29	32	31	30.6666667	1.527525232
	Staphylococcus aureus	30	31	30	30.3333333	0.577350269

The result in table 1.1 can be summarized in the bar chart below (Fig. 1.1) which clearly shows the different zone of inhibition in millimeter. The lengths of the bars represent the diameter of zone of inhibition. The bar diagram, Fig 1.1 provides ease for visual analysis of the results that we obtained after the experiment. The highest zone of inhibition is seen in E.Coli with aqueous extract of canary seed while lowest zone of inhibition is seen in Staphylococcus aureus with ethanolic extract. The average values of zone of inhibition are 22.33mm and 10.33mm respectively. The values stated are in comparison to the standard ciprofloxacin drug which obviously shows greater zone of inhibition.

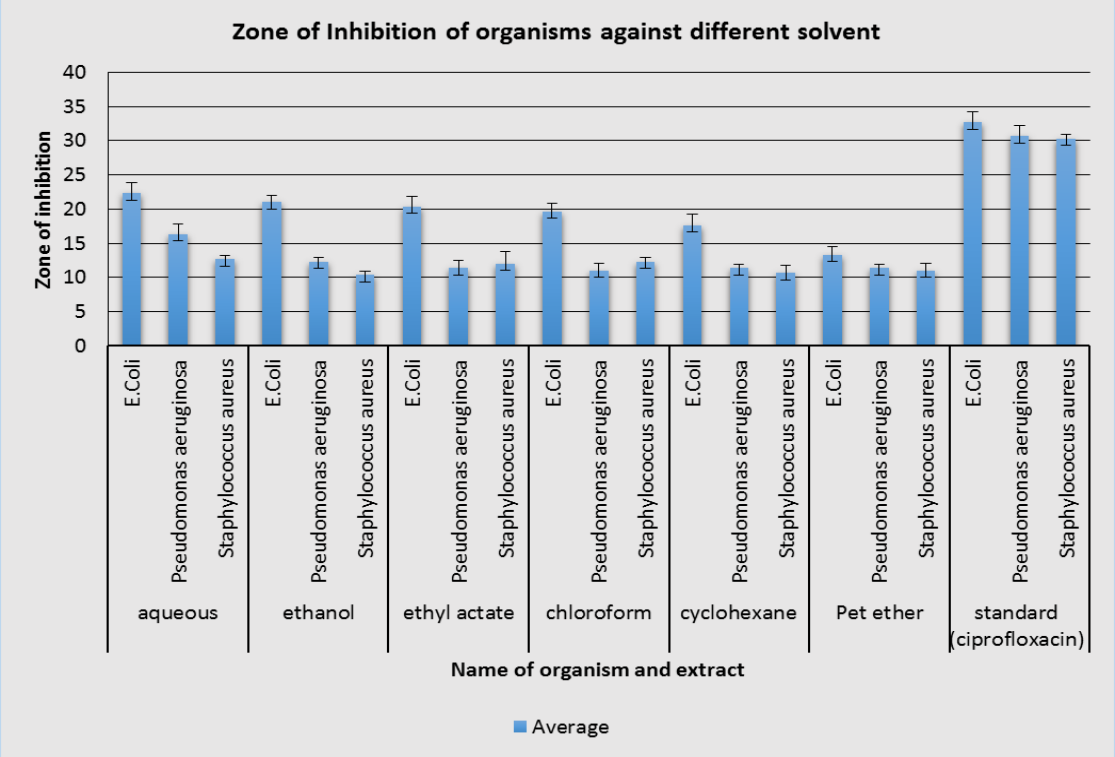

Fig. 1: Graphical representation of zone of inhibition of different organisms in different solvent extract of *Phalaris canariensis*.

Antioxident activity: At a glance, we can see that in all cases, increase in concentration shows increase in percentage of inhibition. Therefore, we can say that concentration is directly proportional to percentage of inhibition. Percentage inhibition for EECS, EAECS and CECS are very close to one another at the concentration 500µg/ml. As concentration is increased, CHECS shows the highest increase in percentage of inhibition compared to the rest. CHECS also shows the second highest percentage of inhibition compared to all of the extracts use in carrying out the experiment. EAECS shows the least increase in the percentage of inhibition, the highest being shown by PEECE.

All these information is summed up in the table 2:

Table 2: Antioxident activity of *Phalaris canariensis* extract dissolved in different solvent.

Conc.(µg/ml)	% of Inhibition					
	AECS	EECS	EAECS	CECS	CHECS	PEECE
500	84.92707	82.33387	80.71313	81.68558	88.9756888	94.4927066
250	80.06483	81.84765	80.06483	76.98541	84.0307942	93.9270665
125	77.14749	77.47164	77.47164	74.23015	81.465154	89.3614263
62.5	68.07131	67.90924	77.63371	71.9611	78.178282	70.2820097
31.25	65.80227	63.53323	76.17504	70.98865	75.6742301	52.3646677
15.625	64.505567	61.26418	75.85089	70.50243	70.6012966	37.0162075
7.813	58.02269	59.80551	72.93355	68.39546	66.2528363	32.2884927
3.906	51.21556	58.02269	72.28525	65.47812	63.7974068	24.6952998
1.953	49.4327	56.7261	70.3404	64.0194	60.0421394	20.9400324
0.977	47.812	52.8363	68.8817	58.5089	53.4489465	19.4846029

The scatter line graph shown in Fig 1.1 provides a visual representation of Table 1.1. From the graph, we can say that PEECE has the most prominent increase in percentage increase as concentration increased and shows an upward curve. The percentage of inhibition is the highest for AECS and lowest for EAECS at concentration 500µg/ml. The graph for EAECS shows very low increase in percentage of inhibition compared to the rest and is less a curve but almost a straight line. AECS and EECS shows similar pattern of increase in percentage of inhibition. For CECS, the graph shows increase in percentage of inhibition in almost linear form after the concentration of approximately 10µg/ml. Also, for CHECS, the graph almost takes a linear pattern after the concentration of approximately 120µg/ml. In graph of AECES, there is a linear increase in percentage of inhibition from concentration of approximately 60 to 130µg/ml and again with a lesser but linear increase from 130 to 250µg/ml. However, the graph slightly falls after this concentration with percentage of inhibition increasing with increasing concentration but in a very little value. The most irregular curve is shown by EAECS where the percentage of inhibition increases as well as decreases randomly as concentration is increased.

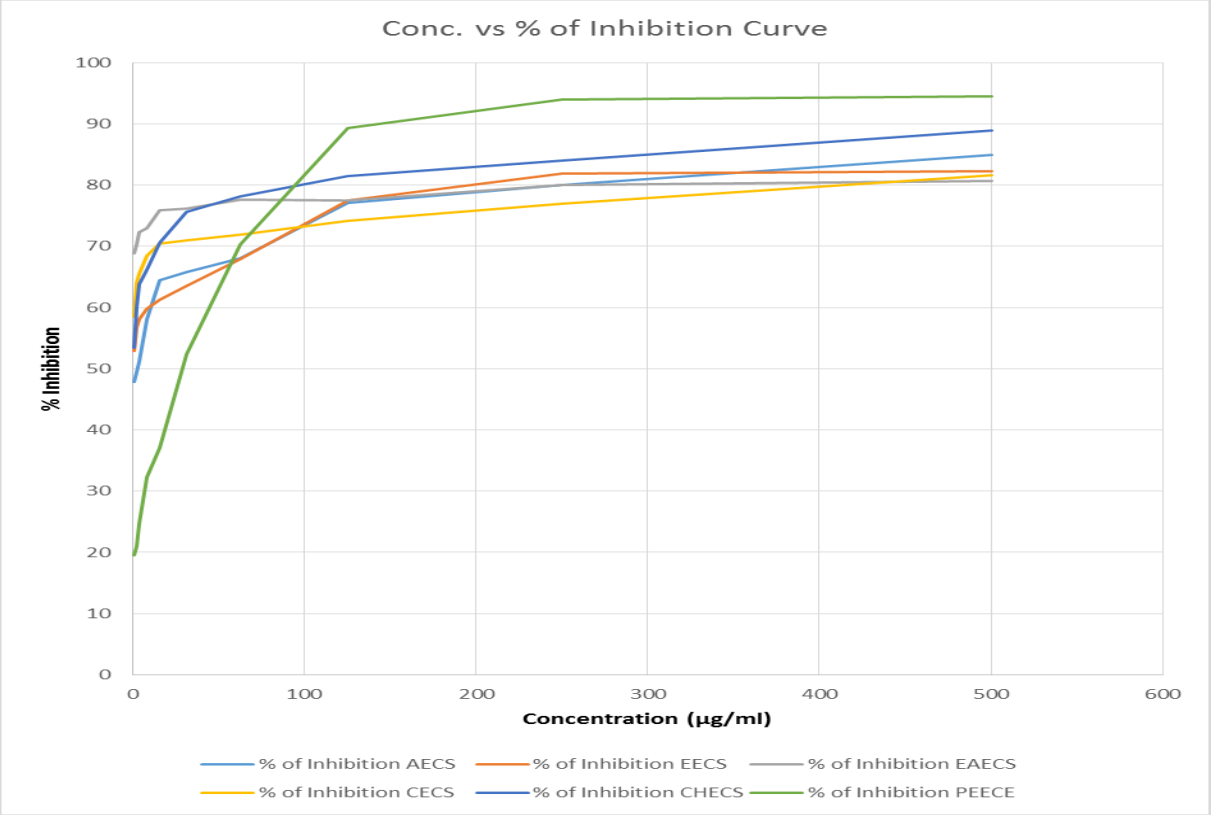

Fig. 2: Graphical representation of concentration against percentage of inhibition of the *Phalaris canariensis* extract dissolved in different solvent.

CONCLUSION

In conclusion we can say that extracts of seeds of *Phalaris canariensis* possess antimicrobial and antioxidant activity. More precisely the acting antimicrobial compound can be a polar compound as it shows highest activity in aqueous extract.

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