ANOVA AND ANTIMICROBIAL ACTIVITY OF FIDDLEHEAD FERN *DIPLAZIUM ESCULENTUM (RETZ.) SW.,* AGAINST HUMAN PATHOGENS

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ABSTRACT

Earlier Jharkhand was a tribal area and the tribals were mainly dependent on agricultural and forest products. Around 130 ethnic tribal communities live in North East India exhibits unique culture, dialect and tradition. Even today for their subsistence and livelihood, they use to collect various edible plants including ferns and fern-allies from wild accessed an inventory survey by Jharkhand, India. One species viz. *Diplazium esculentum (Retz.) Sw*., were directly collected from wild habitat of Lohardaga district, Jharkhand. The methanolic plant extract showed highest anti-bacterial activity (933.365 mm and 462.365 mm zone of inhibition) against *Pseudomonas aeroginosa* and *Escherichia coli* respectively. The acetone and aqueous extract of fiddlehead fern (*Diplazium esculentum*) showed moderate antibacterial activity (148.365 mm and 104.405 mm zone of inhibition) against *Pseudomonas aeroginosa* and *Enterococcus faecalis* respectively. The ethanolic and methanolic extract showed anti-fungal activity (125.6 mm and 66.725 mm zone of inhibition) against *Candida albicans.* Present study focusses on analysis of anti-microbial activity of fiddlehead fern (*Diplazium esculentum*).

Keywords: Fiddlehead fern (*Diplazium esculentum*), Anti-microbial activity and Anova

INTRODUCTION

Diplazium esculentum is probably the most consumed fern which is found throughout Asia and Oceania. It is commonly used by the people of interior and agriculturally difficult terrains living hilly regions such as Himalayas [1]. These Wild edible ferns are the best and suitable alternative to staple crops [2]. The plant is bipinnate with frond 15 cm, pinnae is about 8 cm long and 2 cm wide [3]. Worldwide, many fern species are used in diet and for the medication of several diseases. These edible ferns are rich source of various bioactive compounds and shows anti-inflammatory, anti-cancer and anti-oxidant activities [4]. Hence, ferns can be exploited for the development of cosmetics, nutraceuticals and pharmaceutical products [5].

Fiddlehead ferns are popular among various edible ferns. Different tribal communities of the world such as Himalayan regions of India, Nepal, China and Pakistan consume these ferns in their diet as seasonal leafy vegetables [6]. Succulent and tender young fronds of Fiddlehead ferns are the edible portion and are termed as croziers. The genus 'Diplazium' is a predominant group of fiddlehead ferns and includes a few popular edible species such as *D. esculentum, D. sammatii, D. proliferum* etc. In lower Himalayan regions, *Diplazium esculentum* is a popularly consumed fern, and is locally known as 'Lungru' in western Himalaya and as 'Dheki saag' in North Eastern Himalayan states of India [7]. Since the edible fronds emerge only during the rainy season, the fronds are pickled, dried, and stored for use throughout the year [8].

Diplazium esculentum extracts (Aqueous and organic) showed higher anti-oxidative activities by the ferric thiocyanate (FTC) method and thiobarbituric acid (TBA) method than the positive control α-tocopherol (Vitamin E), [9]. Anti-microbial activity was shown against some human and plant pathogens with tetracycline as standard. Combination of all extracts with tetracycline showed more antibacterial activity than alone [10]. Phytochemical screening is a prerequisite to the antimicrobial activity.[11] or the determination of the concentration of specific phytochemicals in the extract, e.g., total phenolic and flavonoid contents [12, 13]. The presence of phytochemicals in the extracts can be qualitatively characterized by screening process. The bioactivities of most phytochemical classes are available in the literature and will provide an overall estimate of the bioactivity of the extracts of *Diplazium esculentum*.

Recently, several pathogenic microorganisms have developed antibiotic resistance, and these antibiotics can have undesirable side effects [14]. Thus, researchers are focusing on botanicals for the development of herbal-based antibiotic substitutes [15]. Anti-microbial studies performed with *Diplazium esculentum*. Anti-microbial activity was considered good [minimum inhibitory concentration (MIC) less than 100 μg/mL], moderate (MIC from 100 to 500 μg/mL), weak (MIC from 500 to 1000 μg/mL), or inactive (MIC over 1000 μg/mL). Inactive results of anti-microbial activities of *Diplazium esculentum* did not include in this study [16, 17]. The objective of this study was to analyses the anti-microbial function of fiddlehead fern (*Diplazium esculentum*).

MATERIAL AND METHODS

Sample Collection: The sample was collected from the Lohardaga District, Jharkhand. The full-grown plant of Fiddleheads fern (*Diplazium esculentum*). Only healthy leaves of Fiddleheads fern (*Diplazium esculentum*) were collected.

***Solvents Used*:** The plant extracts (*Diplazium esculentum*) are prepared by using the organic solvent. The common structure of organic solvents (at least 1 carbon and 1 hydrogen atom), low molecular weight, volatility and lipophilicity and they occur in liquid firm at room temperature. Secondary metabolites were needed for plants, that is organic in nature and organic solvents is used to dissolve secondary metabolites. On the time of extraction solvents diffuse in to the solid plant material and solubilize compound with similar polarity. The following five solvents was used for plant extraction, first three are organic solvents- Methanol, Chloroform and Hot Water [18].

***Pathogens Used*:** The analysis of anti-microbial activity of fiddlehead fern (*Diplazium esculentum*) was performed against seven microbial strains such as *Streptococcus mutants, Es cherichia coli, Enterococcus faecalis, Bacillus subtilus, Pseudomonas aeroginosa, Lactobacil lus acidophilus* and *Candida albicans.*

***Agar Disc Diffusion method*:** The agar disc diffusion is best method to analyze the anti-microbial activity against numerous microorganisms. The zone of inhibition measures the diameter in mm [19].

***Incubation & determination of Zone of Inhibition (ZOI)*:** Loading the sample (*Diplazium esculentum*), after that the plates were incubated in straight position for 24 hrs. at 37°C for

bacterial growth. After that plate are observed for measuring the zone of inhibition and calculate the MIC (Minimum Inhibitory Concentration) value.

RESULTS AND DISCUSSION

A total five different solvent extracts (Aqueous, Methanol, Acetone, Ethanol and Dimethyl Sulfoxide) were used against a series of microbial strains including *Streptococcus mutants, E scherichia coli, Enterococcus faecalis, Bacillus subtilus, Pseudomonas aeroginosa, Lactobacillus acidophilus* and *Candida albicans.* The methanolic extract of *Diplazium esculentum* leaves has been evaluated for anti-bacterial activity by using the disc diffusion method [20].

The methanolic leaf extract showed highest anti-bacterial activity (933.365mm and 462.365 mm zone of inhibition) against *Pseudomonas aeroginosa* and *Escherichia coli* respectively. The acetone and aqueous extract of fiddlehead fern (*Diplazium esculentum*) showed moderate anti-bacterial activity (148.365mm and 104.405 mm zone of inhibition) against *Pseudomonas aeroginosa* and *Enterococcus faecalis* respectively (Table 1 and Graph 1).

The anti-fungal activity of fiddlehead fern (Diplazium esculentum) leaves against three fungal strains using the agar diffusion method has been reported [17]. The ethanolic and methanolic extract showed anti-fungal activity (125.6mm and 66.725mm zone of inhibition) against *Candida albicans* (Table 1 and Graph 1).

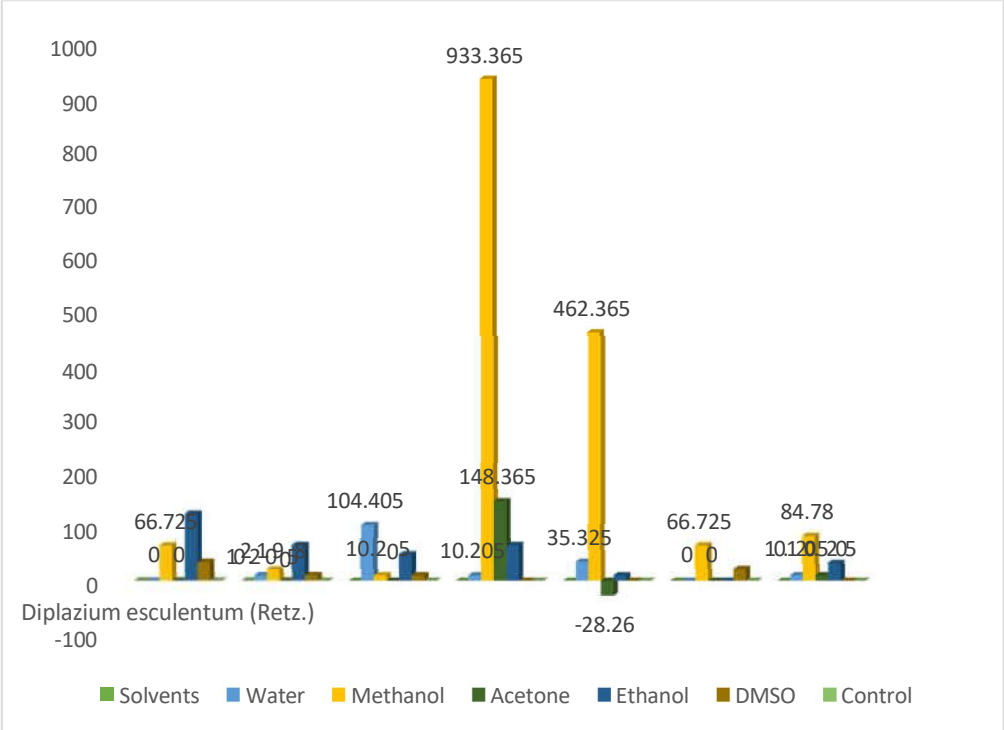
ANOVA shows that as group size and within fiddlehead fern extract relatedness increased monotonically, so did anti-microbial strength (Spearman's rank correlation $p < 0.05$ for MIC100). P value = 0.000 (calculated) is less than .05 (confidence interval). As we have strong evidence to support that we can reject null hypothesis. There is a significant difference between the five solvents groups. So, it can be concluded that at least one of the means is different in the given one group. P value of solvents in the given group of ANOVA report is 0.038713 respectively (Table 2).

Figures 1. Fiddlehead fern (*Diplazium esculentum*), plants and fiddlehead plants extract

Fiddlehead fern inhibition on ***Candida albicans*** in different solvents					
Solvents	r_1 (mm)	Area of disc (mm^2)	r_2 (mm)	Area of inhibition (mm^2)	Zone of inhibition (mm^2)
Water	3	28.26	0	0	0
Methanol	3	28.26	5.5	94.985	66.725
Acetone	3	28.26	0	0	0
Ethanol	3	28.26	7	153.86	125.6
DMSO	3	28.26	4.5	63.585	35.325
Control	3	28.26	0	0	0
Fiddlehead fern inhibition on ***Bacillus subtilis*** in different solvents					
Solvents	r_1 (mm)	Area of disc (mm^2)	r_2 (mm)	Area of inhibition (mm^2)	Zone of inhibition (mm^2)
Water	3	28.26	3.5	38.465	10.205
Methanol	3	28.26	4	50.24	21.98
Acetone	3	28.26	0	0	0
Ethanol	3	28.26	5.5	94.985	66.725
DMSO	3	28.26	3.5	38.465	10.205
Control	3	28.26	0	0	0
Fiddlehead fern inhibition on ***Enterococcus faecalis*** in different solvents					
Solvents	r_1 (mm)	Area of disc (mm^2)	r_2 (mm)	Area of inhibition (mm^2)	Zone of inhibition (mm^2)
Water	3	28.26	6.5	132.665	104.405
Methanol	3	28.26	3.5	38.465	10.205
Acetone	3	28.26	0	0	0
Ethanol	3	28.26	5	78.5	50.24
DMSO	3	28.26	3.5	38.465	10.205
Control	3	28.26	0	0	0
Fiddlehead fern inhibition on ***Pseudomonas aeruginosa*** in different solvents					
Solvents	r_1 (mm)	Area of disc (mm^2)	r_2 (mm)	Area of inhibition (mm^2)	Zone of inhibition (mm^2)
Water	3	28.26	3.5	38.465	10.205
Methanol	3	28.26	17.5	961.625	933.365
Acetone	3	28.26	7.5	176.625	148.365
Ethanol	3	28.26	5.5	94.985	66.725
DMSO	3	28.26	0	0	0
Control	3	28.26	0	0	0
Fiddlehead fern inhibition on ***Escherichia coli*** in different solvents					
Solvents	r_1 (mm)	Area of disc (mm^2)	r_2 (mm)	Area of inhibition (mm^2)	Zone of inhibition (mm^2)
Water	3	28.26	4.5	63.585	35.325
Methanol	3	28.26	12.5	490.625	462.365

Acetone	3	28.26	0	0	-28.26
Ethanol	3	28.26	3.5	38.465	10.205
DMSO	3	28.26	0	0	0
Control	3	28.26	0	0	0
Fiddlehead fern inhibition on ***Lactobacillus acidophilus*** in different solvents					
Solvents	r_1 (mm)	Area of disc (mm^2)	r_2 (mm)	Area of inhibition (mm^2)	Zone of inhibition (mm^2)
Water	3	28.26	0	0	0
Methanol	3	28.26	5.5	94.985	66.725
Acetone	3	28.26	0	0	0
Ethanol	3	28.26	0	0	0
DMSO	3	28.26	4	50.24	21.98
Control	3	28.26	0	0	0
Fiddlehead fern inhibition on ***Streptococcus mutans*** in different solvents					
Solvents	r_1 (mm)	Area of disc (mm^2)	r_2 (mm)	Area of inhibition (mm^2)	Zone of inhibition (mm^2)
Water	3	28.26	3.5	38.465	10.205
Methanol	3	28.26	6	113.04	84.78
Acetone	3	28.26	3.5	38.465	10.205
Ethanol	3	28.26	4.5	63.585	35.325
DMSO	3	28.26	0	0	0
Control	3	28.26	0	0	0

Table 1: Fiddlehead fern shows zone of inhibition on microbial strains in different solvents.



Graph 1: Shows anti-microbial activity (Zone of inhibitions)

Anova: Single Factor						
SUMMARY						
Groups	***Count***	***Sum***	***Average***	***Variance***		
Water	7	170.345	24.335	1385.89		
Methanol	7	1646.145	235.1636	119086.3		
Acetone	7	130.31	18.61571	3414.855		
Ethanol	7	354.82	50.68857	1761.758		
DMSO	7	77.715	11.10214	179.4095		
Control	7	0	0	0		
ANOVA						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit***
Between Groups	277741.308	5	55548.26	2.648767	0.038713	2.477169
Within Groups	754969.1151	36	20971.36			
Total	1032710.423	41				

Table 2: ANOVA test report of Solvents

In the present investigation study, the efficacy and response of ethanol to specific bioassays were checked over that of methanol which is more toxic and also can extract more phytochemicals. although the latter can extract a broader range of phytochemicals [21]. Fiddlehead fern (*Diplazium esculentum*) contains anti-microbial, anthelmintic, and anti-diarrheal activities and more specific bioassays should be conducted for the validation and assessment of the potential functions of the plants in human systems. Cardiac glycosides are toxic due to its effect on the heart and atrial fibrillation [22]. It is important to note that the presence of cardiac glycosides was observed in *Diplazium esculentum* but undetected when aqueous was used. The exact levels of these compounds need to be ascertained because of the danger posed to humans by overconsumption of the plant as food. The screening also showed the absence of cyanogenic glycosides that are much more toxic than the cardiac counterpart [23]. Their absence in *Diplazium esculentum* indicates no adverse effects of cyanide poisoning associated with cyanide poisoning when ingested and metabolized by humans.

Ethanol, aqueous, and petroleum ether extract (10, 25, and 50 mg/mL), of Rhizome of *Diplazium esculentum* evaluated for their anti-helmintic activity against *Pheretima posthuma* and demonstrated significant results. Ethanolic extract showed the highest activity The difference in phenolic and flavonoid content of the extracts may be attributed to the solvent used on extraction. Phenolic compounds, in general, inhibit various types of oxidizing enzymes. The diverse biological roles of phenolic compounds in *Diplazium esculentum* leaves create opportunities for searching specific phytochemicals with their corresponding health benefits. There is a requirement of further studies with other solvents for the extraction of some more phytochemicals present in *Diplazium esculentum* that possess free radical scavenging activity and other promising health benefits.

CONCLUSIONS

The methanolic plant extract showed the highest anti-bacterial activity (933.365 mm and 462.365 mm zone of inhibition) against *Pseudomonas aeroginosa* and *Escherichia coli* respectively. Moderate anti-bacterial activity (148.365 mm and 104.405 mm zone of inhibition) against *Pseudomonas aeroginosa* and *Enterococcus faecalis* by acetone and aqueous extract of the experimental plant. The ethanolic and methanolic extract showed anti-fungal activity with 125.6 mm and 66.725 mm zones of inhibition against Candida albicans. *Diplazium esculentum* is an edible fern, rich in several nutritional characteristics like commonly consumed vegetables. Omega 6 fatty acid di-homo-gamma linolenic acid and polyphenols present their potential nutraceutical applications. Acceptable sensory scores of the soup prepared with *Diplazium esculentum* biomass also indicate its suitability for instant food production and inclusion in existing food baskets. The outcome of this study gives an idea by demonstrating the prospective benefits of the fern species and promoting its utilization, domestication, and preservation as a popular food.

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