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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING AND COMPARATIVE STUDY OF ANTIBACTERIAL ASSAYS OF *Carumroxburghianum AND Trigonellafoenum-graecum* OBTAINED FROM PLANT SEEDS

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ABSTRACT

To control the infections and microbial food spoilage, the application of synthetic antibiotics can be replaced by antimicrobial agents from natural sources. Therefore, present study was done to explore nontoxic antimicrobial agents from two enormous plants named *Carumroxburghianum* (radhuni) and *Trigonellafoenum-graecum* (fenugreek) through antibacterial and phytochemical assays. In the current work, the crude extracts of radhuni andfenugreek were collected using ethanol and methanol. Antibacterial effects of both extracts taken of second, fifth and seventh days were observed against selected eleven bacteria. Extracts of different days had different effects on six bacteria. Radhuni showed positive results against *Shigellaflexineri*, *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus subtilis, Bacillus cereus* and *Streptococcuspneumonae* and fenugreek showed positive results only for *Bacillus cereus*. The best result was from ethanol extracts of both seeds, whereas Radhuni from the fifth day against *Bacillus subtilis* and fenugreek from the seventh day against *Bacillus cereus*. The zone of inhibition was greater for radhuni than fenugreek against *Bacillus cereus*. The high activity index of ethanol extract at day five against *Shigellaflexineri* indicated high sensitivity to the extract. Phytochemical assays like tests for alkaloids, terpenoids, tannins, saponins, steroids, phenolic compounds, flavonoids and cardiac glycosides were performed on both seed extracts to identify presence of secondary metabolites. The results from this research can be significant in terms of exploring antimicrobial properties of both extracts especially radhuni. This study could be the beginning of discovering such novel and less expensive microbial agents against various bacterial species.

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INTRODUCTION

Spices have been used for not only flavor and aroma of the foods but also to endow with antimicrobial properties to treat chronic as well as infectious diseases (Nanasombat *et al*., 2002). Such a spice is *Carumroxburghianum* which is erect and usually much branched herbaceous annual or biennial plant growing 30 – 100 cm tall having small seeds of 1 to 1.5mm long and brown in color (Khalsa *et al.,* 2008). It is commercially known as 'Radhuni' in Bengali and close relative to Ajwain and in English it is called similar to 'Wild celery'. It is native to tropical Asian countries such as India, Pakistan, China, and Indonesia, some regions of Africa and also cultivated in Bangladesh (Minosuke, 1958; Heinrich *et al*., 2003; Solomon *et al.,* 2011). It is a spice which is strongly aromatic and medicinal plant that contains 1.8-2% essential oil. The essential oil, Radhuni oil, or its emulsion in water, is the main constituent of "grip water" and is considered to be aromatic, carminative especially useful in flatulence, collie pain, anti-diarrheal, anti-tumor, anti-oxidant, CNS, diuretic, cardiovascular, hypotensive, vomiting, spasmolytic and hiccups of infants and children (Khan *et al.,* 2012). Due to all these benefits, it is one of the important spices used in traditional medicine. But still various other modern medicinal properties are not extensively known and hence mostly used in the preparation of Ayurvedic medicine. *Trigonellafoenum-graecum* is another erect annual herbaceous plant growing 20-50 cm (up to 100 cm) long, survives only one growing season. The seed is small, 2 to 3mm long and yellowish golden in color with a strong odor. The plant falls under the family of Fabaceae (Leguminosae) and indigenous to the eastern shores of the Mediterranean Sea, but it is grown in South Asian countries like India, Pakistan and Bangladesh, Morocco, Southern France, Lebanon, Egypt and England (Flammang *et al*., 2004). Commercially it is known as 'Methi' in Bengali and in English it is called 'Fenugreek'. Fenugreek herb's seeds acquire toxic oils and other bioactive constituents comprise volatile oils and alkaloids in it have been shown to be toxic to bacteria parasites and fungi. The fenugreek seeds are loaded

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with dietary fiber that can lower blood sugar levels in diabetes. Moreover, it is extensively used as a galactagogue that is often used to boost up production of milk in lactating women and to cure breast cancer (Sherif *et al*., 2015). Fenugreek seed is useful for tuberculosis, diabetes, atherosclerosis, constipation, high cholesterol, hyper triglyceridemia and externally it is used as a poultice for abscesses, boils, carbuncles, etc (Sahay *et al.,* 1994). Gastric ulcers can be handled through fenugreek seeds as it sooths the gastro intestine tract. The cleansing process of fenugreek makes it a precious plant as it helps purify blood, cleaning lymphatic system, and detoxify the body. Moreover, the seed oil acts as an emollient and makes pores and skin smoother and soft. In ailments like hay fever and sinusitis it can be used. The seeds are considereduseful in heartsickness and aphrodisiac and as a galactogogue promoting lactation in breast feeding mother and help in breast cancer (Tiran, 2003). The smallpox patients are also given an infusion of seeds as a cooling agent. As a natural health product, it is competent of treating and curing diseases for which, it has been well thought-out as a potential nutraceutical (Kalra, 2003). Due to all these reimbursements, it is one of the most practiced and oldest traditional medicinal plants in Ayurveda (Basch *et al*., 2003).

Countless of the food-borne pathogens are escalating defiant against antibiotics. With the rise in the emergence of various multidrug resistant microorganisms and the scenario worsening through the indiscriminate use of antibiotics, new and/or alternative antimicrobial compounds must be developed to treat general infections. With the changing patterns of susceptibility and the availability of new antimicrobial agents, continuous updating of knowledge concerning treatment of disease caused by such pathogens is required (Khan *et al*, 2010). Plants have a broad range of secondary metabolites such as tannins, alkaloids and flavonoids, saponins which have been bring into being to have antimicrobial properties *in vitro* (Khan *et al.*, 2009). For controlling infections and microbial food spoilage, using chemical or synthetic antimicrobial agents is one of the ancient methods. Patrons are more conscious about the safety of foods containing chemical preservative. There is growing evidences about adverse effects of chemical preservatives on human health, so continuous pressure has been developed to trim down the quantity of added preservatives in foods (Bukvicki*et al*., 2014). Therefore, there has been immense interest in the development of efficient and nontoxic antimicrobial compounds from natural sources, such as extracts of plants, for food preservation (Shan *et al*., 2007).

The antibacterial activities of the cold aqueous, hot aqueous, ethanol, methanol and acetone extracts of *Carumroxburghianum* (Radhuni) extracts were tested against two pathogenic bacteria namely *Serratiamarcescens* and *Bacillus cereus* and one yeast, *Rhodotorulamucilaginosa* by well diffusion method and the finding suggest positive activity against all the species (Dhiman *et al.*, 2015). In another study, the antimicrobial activity of essential oil of *Carumroxburghianum* was determined by using Agar Diffusion Method and was checked on six bacteria's. These were *Bacillus subtilis, Bacillus licheniformis, Escherichia coli, Micorococcusluteus, Nocardiaasterides* and *Salmonella typhimurium* from which *Bacillus subtilis, Nocardiaasterides* and *Salmonella typhimurium*showed positive result and rests showed negative activity (Iqbal *et al.,* 2014). There is an extensive similar another spice which is very close relative of *Carumroxburghianum* and most of the researches have been conducted with that spice named '*CarumCopticum'*. The oil of this plant also is able to inhibit the growth of this parasite (Alizadeh *et al.*, 2010). Another study was conducted with aqueous, ethanol, benzene, chloroform, hexan and petroleum ether extracts of fenugreek and antimicrobial activity was done with agar well diffusion method against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*and *Klebsilla* and showed negative results for all (Al- abdeen *et al.*, 2010). With the intension of developing modern novel therapeutics, from last few decades scientists have been focusing on natural extracts to assess the antimicrobial properties. A number of plant systems have been studied by the researchers which exhibited antimicrobial action. Among diverse ranges of herbal extracts, radhuni (very less studied) and fenugreek (more studied) are also two of the candidates that have been tested for its activity against ample range of microorganisms like bacteria, virus, and fungus. Sensitivity of the extracts towards bacteria is reliant not only on the solvent system but also on the type of microorganisms used in the experiments. To scrutinize antibacterial effect of radhuni and fenugreek extracts eleven different bacteria strains were selected which includes: *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, Klebsiella *sp.*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigellaflexinera*, *Staphylococcus aureus*, *Streptococcus pneumonae*, *Enterobacter cloacae* and *Aeromonushydrophila.* Moreover, to examine the existence of alkaloids, terpenoids, tannins, saponins, steroids, phenolic compounds, flavonoids and cardiac glycoside in both radhuni and fenugreek; the phytochemical assay was demonstrated as the medicinal plants are valuable for healing and curing of human diseases because of the existence of phytochemical constituents. Nevertheless, several synthetic drugs or antibiotics against such microorganisms have manufactured by numerous pharmaceuticals and other medicinal companies but since many eras the escalating number of multi drug resistant bacteria (MDR) is becoming immense concern in fighting comprised health status in medicine world. Laying in such situation; different parts of plants like seeds of radhuni and fenugreek posses' some antimicrobial properties and phytochemical constituents which can definitely light a hope not only in the treatment of infections rooted by bacteria but also in constructing novel antibiotics extorted from these products of nature. Therefore, the aim of the study was to examine the antimicrobial activities of radhuni and fenugreek against eleven selected bacteria; on the ethanol and methanol extracts and to ensure the existence of secondary metabolites by executing a number of phytochemical assays.

MATERIALS AND METHODS

Collection and Reference of Materials

Both of the seeds; radhuni and fenugreek were bought from local market of Dhaka city. All the reagents, chemicals and apparatus being used were from Microbiology Specialized Research Laboratory, Department of Mathematics and Natural Sciences; where few reagents were prepared fresh within the laboratory only. During the research; clinical strains of selected eleven bacteria and several antibiotics were used which were taken from the conserved bacterial stock in the departmental laboratory.

Processing of the Seeds

Both of the seeds were processed exactly in a same procedure during the whole study. Seeds were sundried for 5-7 days and

then to make powder of seeds without any impurities; grinder was used and stored the seeds powder in air tight box separately at the laboratory at room temperature for further works.

Extraction procedure

Two types of extraction were done with the radhuni and fenugreek seed powder; extracted in ethanol and methanol.

Ethanol

Radhuni powder (20 gm) was extracted with ethanol (100 ml) for 2 days, 5 days and 7 days separately by conventional method. After extraction the sample was filtered with filter paper (Whatman No. 1) and then concentrated in a water-bath at about 80°C temperature until the contents of the beaker became pourable into a petri-dish that is about 20 ml and then put it back in the water-bath to obtain oily sticky substances which is the product of interest. It was then kept in a vial, labeled and stored below 10 ºC in the refrigerator for further use. The whole process was repeated multiple times for the collection of a substantial amount of extracts for the study.Just like this ethanol extraction process of radhuni seeds, fenugreek seed extract in ethanol was done independently.

Methanol

Radhuni powder (100 gm) was extracted with ethanol (500 ml) for 2 days, 5 days and 7 days separately by conventional method. After extraction the sample was filtered with filter paper (Whatman No. 1) and then concentrated in a water-bath at about 80°C temperature until the contents of the beaker became pourable into a petri-dish that is about 20 ml and then put it back in the water-bath to obtain oily sticky substances which is the product of interest. It was then kept in a vial, labeled and stored below 10 ºC in the refrigerator for further use. The whole process was repeated multiple times for the collection of a substantial amount of extracts for the study.Just like this methanol extraction process of radhuni seeds, fenugreek seed extract in methanol was done discretely.

Antimircobial Assay

Agar or Well Diffusion Method

This is the method used in whole research for accomplishing antimicrobial tests. The traditional method is obtaining the previously lawned nutrient agar plate and aseptically punching holes in the agar using a sterile cork borer but in this experiment the holes were created by the back of an autoclaved micropipette tip. Then marking of the holes were done to pour the samples of seed extracts (radhuni and fenugreek) according to the further works by means of separate autoclaved micropipettes. After that, to compare the result; an appropriate positive control of an antimicrobial disc was placed on each plate and at 37°C temperature for 24 hours the plates were incubated. Next, the positive result for antimicrobial tests depended on the existence of a clear zone around the hole. The entire method was replicated discretely for each eleven organisms for both radhuni and fenugreek seed's ethanol and methanol extracts.

Measuring the Inhibition Zone

The zone of inhibition means the width of clear zone around the well and antibiotic disk. It is the most decisive part of the study which (clear zone) was measured three times in millimeter (mm) with a ruler or scale and was noted down to find the average of the three values which was recorded as well.

Measuring the Activity Index

The activity index of the positive results holding methanolic and ethanolic extracts of radhuni and fenugreek were measured by using the following formula:

Activity Index (AI) = Zone of inhibition of extract/ Zone of inhibition of antibiotics

Phytochemical Screening

In the study, for both radhuni and fenugreek seeds; eight different kinds of phytochemical assays were accomplished individually and exactly with the same process. These were for alkaloids, terpenoids, tannins, saponins, steroids, phenolic compounds, flavonoids and cardiac glycoside.The stock solution for both radhuni and fenugreek were prepared using powdered samples. The powder was taken into 100 ml of distilled water. This was boiled for about 10 minutes and was filtered while still hot; filtrate was left to cool down and used to perform further tests in the test tubes.

Tests for Alkaloids: A few drops of picric acid were added slowly to 0.5 ml of filtrate. The presence of alkaloids was confirmed by white or creamy precipitation

Tests for Terpenoids: Exactly 2ml of chloroform was added in 5ml of filtrate and then 3 ml of concentrated sulfuric acid was incorporated that forms a layer. The presence of terpenoids in the filtrate was positively indicated by observing reddish brown precipitates at the interface between the two layers

Test for Tannins: For the test of tannin, 1 ml of filtrate was diluted with 5ml of distilled water and five to six drops of10% of ferric chloride is added to it. The presence of tannins in the filtrate was positively indicated by formation of brownish-green or bluish-black precipitate

Tests for Saponins: Filtrate 2.5ml of was diluted with 10ml of distilled water and shaken robustly for 2 minutes. The presence of saponins in the filtrate was positively indicated by visibility of frothing in the tube.

Tests for Steroids: For steroids, 2 ml of chloroform and 2 ml of sulphuric acid were added gradually to 2 ml of filtrate. The presence of steroids was confirmed by producing red color in the lower chloroform layer.

Tests for Phenolic Compounds: A5% solution of ferric chloride was added to 5 ml of filtrate. The presence of phenolic compound was positively indicated by turning the extract into dark green color.

Tests for Flavonoids: A few drops of 20% sodium hydroxide solution are added in 1 ml of filtrate. The presence of flavonoids in the extract is positively indicated by a change of color to yellow. Then acid was added and extract turning back to its native color reconfirmed the test result.

Tests for Cardiac Glycoside: For this test, 2ml of glacial acid including 1 drop of ferric chloride solution; was added to 5ml of filtrate. Then gradually by the side of the test tube; 1 ml of sulphuric acid was added. The presence of cardiac glycoside (deoxysugar characteristics) in the extract was confirmed by formation of a brown ring at the interface. Moreover, in the glacial acid layer; a violet or greenish ring may be seen below that ring.

RESULTS

Antibacterial Assay

As mentioned earlier; eleven different bacteria were used for analyzing the antimicrobial properties of ethanolic and methanolic extracts of radhuni and fenugreek. Extracts of radhuni showed noteworthy positive results against six microbes which were *S. flexineri, S. typhi, S. aureus, B. cereus, B. subtilis*and *S. pneumonae* (Table 1) whereas extracts of fenugreek showed positive result only against one bacterium that was *B. cereus* (Table 2); from the eleven test microbes and negative or non descriptive results were given by rest.Inradhuni seed extracts, it was observed that the average zone of inhibition for all the six microbes – *S. flexineri, S. typhi, S. aureus,B. cereus, B. subtilis*and *S. pneumonae*–was maximum for ethanol from which *B. subtilis*showed highest average zone of inhibition that is 20.20 mm for 5th day extract of *B. subtilis* and unremarkable result or lowest average zone of inhibition was for methanol that is 0.00 mm from the 2th day extract of *S. typhi* and *S. pneumonae.*

Comparison between Positive Antibacterial Activities of Radhuni and Fenugreek

The antibacterial effect of ethanol and methanol extracts of radhuni and fenugreek was shown against only one bacterium *Bacillus cereus* through its zone of inhibition within all eleven tested microbes. So by comparing both seed's ethanol and methanol extracts; radhuni showed maximum zone of inhibition in all three replicates.Positive antimicrobial effects (average zone of inhibition) with positive controls,produced by ethanol and methanol extracts of radhuni and fenugreek.

Comparison of Activity Index

The following formula is used for both radhuni and fenugreek seeds' ethanol and methanol extracts to calculate the activity index values.

Activity Index (AI) = zone of inhibition of extracts/ zone of inhibition of the antibiotics

For radhuni seed extracts, among six microbes the activity index (AI) values for *B. cereus* and *S. pneumonae* was maximum in ethanol extract from the 7th day where rest four were maximum in ethanol extract from the 5th day. From all the AI values, the 5th day ethanol extract of *S. flexineri* has got the highest AI value. For fenugreek seed extracts, the activity index (AI) value for *B. cereus* was maximum in methanol extract from the 7th day.

Phytochemical Assay

For testing various phytochemicals found in both seed extracts, screening was done using various chemicals and reagents. The crude extracts were tested for the presence or absence of secondary metabolites such as alkaloids, terpenoids, tannins, saponins, steroids, phenolic compounds, flavonoids and cardiac glycoside. It turned out that it contained all the phytochemicals except alkaloids.

DISCUSSION

The unrestrained employ of chemical preservatives in food industry over the decades has led to emergence of microbial resistance to classic antimicrobial agents which has become a severe health concern (Kiessling *et al*., 2002). The rising inclinations of multidrug resistance within copious groups of microorganisms against diverse classes of antibiotics led loads of researchers to build up competent drugs from plant sources to defy multidrug resistant strains. For the development and synthesis of novel antimicrobial agents; different parts of plants have created a great contribution to human health and well-being. It has been found that there is correlation between antimicrobial efficacy and amount of phytochemicals present in spices. Radhuni and fenugreek are such spices that come from the plant that falls under the family Apiaceae and Fabaceae respectively; which have loads of restorative properties other than simply being used as household cooking or flavoring agents. *Carumroxburghianum* (radhuni) is used in conventional system of medicine to treat diarrhea, abdominal spasm (colic), asthma, bronchitis cough, common cold, dyspepsia, lethargy, loss of consciousness, palpitation, vomiting, pain in bladder and kidneys as well as considered useful as anthelmintic, antigout, antimicrobial, cardiotonic, carminative, condiment, digestive, diuretic, emmenagogue, stimulant and stomachic (Khan *et al.,* 2012). The other seed *Trigonellafoenum-graecum* (fenugreek) also has a long history of traditional medical uses in Ayurvedic and Chinese medicine and has been used for numerous indications, including labor induction, aiding digestion, hypoglycemic and anti-hyperlipidemic properties, lowers blood pressure, relieves congestion, reduces inflammation and fights infection, contains natural expectorant properties and an excellent source of selenium (Sheikhlar, 2013). For all these vast therapeutic properties, both of these seeds can be the products of interest in the research area to work with.

A study from Pakistan raveled the positive antimicrobial effect of essential oil of radhuni seed on three bacteria *Bacillus subtilis, Nocardia asterides* and *Salmonella typhimurium*out of tested six microbes which were *Bacillus subtilis, Bacillus lichneformis, Escherichia coli, Micorococcus luteus, Nocardia asterides* and *Salmonella typhimurium.* The extract was carried out through hydrodistillation process and antimicrobial assay was determined by using agar diffusion method (Iqbal *et al.,* 2014). In this research, using agar diffusion method, the zone of inhibition was also found for both ethanol and methanol extracts of day 2, 5 and 7 where highest was for *Bacillus subtilis* from the ethanol extract of 5th day and lowest was for *Salmonella typhimurium* from the ethanol extract of 2nd day where *Escherichia coli* did not show any zone of inhibition which represents the exact coherence with the research done by Iqbal*et al.* But the extraction process was different here, where extraction was done via conventional crude method using solvents like ethanol and methanol. Another research finding indicated positive results against two bacteria namely *Serratiamarcescens*, *Bacillus cereus* and one yeast, *Rhodotorulamucilaginosa* where the radhuni seed extraction was done in the cold aqueous, hot aqueous, ethanol,

Table 1. Positive antimicrobial effects (average zone of inhibition) with positive controls, produced by ethanol and methanol extracts of radhuni

Extraction days		2nd day	5th day	7th day	Positive controls
Solvents	Ethanol				
Zone of Inhibition (mm)	*Shigellaflexinera*	17.67 ± 0.470	18.60 ± 0.530	18.44 ± 0.513	17.90 ± 0.173
	Salmonella typhi	12.40 ± 0.529	18.04 ± 0.115	16.07 ± 0.231	28.17 ± 0.289
	Staphylococcus aureus	14.34 ± 0.153	17.34 ± 0.231	17.20 ± 0.173	22.14 ± 0.115
	Bacillus cereus	16.57 ± 0.321	19.50 ± 0.173	19.74 ± 0.115	28.77 ± 0.231
	Bacillus subtilis	14.40 ±0.458	20.20 ± 0.361	18.27 ± 0.231	24.00 ± 0.00
	Streptococcus pneumonae	13.60 ±0.529	15.67 ± 0.115	15.84 ± 0.289	29.07 ± 0.115
Solvents	Methanol				
Zone of Inhibition (mm)	*Shigellaflexinera*	16.94 ± 0.833	18.77 ± 0.681	17.40 ± 0.529	18.20 ± 0.346
	Salmonella typhi	0.00 ± 0.00	17.80 ± 0.346	15.86 ± 0.416	28.07 ± 0.115
	Staphylococcus aureus	14.23 ± 0.950	17.06 ± 0.503	16.23 ± 0.879	22.00 ± 0.00
	Bacillus cereus	11.70 ± 1.044	18.84 ± 0.289	19.03 ± 0.416	28.08 ± 0.115
	Bacillus subtilis	10.20 ± 0.173	19.87 ± 0.416	17.54 ± 0.611	24.07 ± 0.115
	Streptococcus pneumonae	0.00 ± 0.00	17.80 ± 0.346	14.84 ± 0.289	28.70 ± 0.577

Table 2. Positive antimicrobial effects (average zone of inhibition) with positive controls, produced by ethanol and methanol extracts of fenugreek

Extraction days		2nd day	5th day	7th day	Positive controls
Solvents	Ethanol				
Zone of Inhibition (mm)	*Bacillus cereus*	17.07 ± 0.513	18.54 ± 0.651	20.47 ± 0.252	29.08 ± 0.115
Solvents	Methanol				
Zone of Inhibition (mm)	*Bacillus cereus*	18.00 ± 0.889	11.87 ± 0.416	19.10 ± 0.265	25.07 ± 0.115

Table 3. Biochemical analysis of crude extracts of *Carumroxburghianum*and *Trigonellafoenum-graecum*

Biochemicals	Extracts	
	Carumroxburghianum(Radhuni)	*Trigonellafoenum-graecum*(Fenugreek)
Alcaloids	-	-
Terpenoids	+	+
Tannins	+	+
Saponins	+	+
Steroids	+	+
Phenolic compounds	+	+
Flavonoids	+	+
Cardiac glycoside	+	+

+ = Presence, - = Absence

methanol and acetone where hot and cold aqueous extracts of plants possessed less antimicrobial activities in comparison to organic extracts (Dhiman *et al.,* 2015). In this study both ethanol and methanol extracts showed positive result against *Bacillus cereus* as well which is similar to the findings of Dhiman *et al*.

In this study, eleven bacteria were tested from that against six pathogenic bacteria both the ethanol and methanol crude extract of radhuni seed gave positive results for those five bacteria *Bacillus subtili*s, *B. cereus, Pseudomonas aeruginosa*, *Salmonella typhi*, and *Staphylococcus aureus* which are common with this research and showing positive response but *Escherichia coli* was resistant here which is contradictory to the study done by Bhuiyan *et al.* The variation in extraction process, media and bacterial strain could be some of the several reasons for this. In addition, antifungal effects of radhuni seed extracts were not in the purview of this study, but may be considered by other researchers. A research indicated potential antimicrobial activity of radhuni seed extracts by hydro- distillation, against against *S. Aureus* and *E.coli* except *P.aeruginosa* (Chansakaow *et al,* 2014) where in this study; zone of inhibition is found in *P.aeruginosa* along with *S. aureus* except *E.coli* and the extracts were in methanol and ethanol. Although, very less research has been done so far with the radhuni seeds, there are numerous researches that have been conducted with another seed which is very closely related to radhuni named 'ajwan'.

The result of phytochemical screening of ajwan can be compared with phytochemical assay of radhuni seed extract in this study. Different phytochemicals have been found to acquire different functions for which it was necessary to do phytochemical assay of radhuni seed extract in this study. In a research '*CarumCopticum'*(ajwan) seed extract was done with the help of Soxhlet apparatus in different solvents included petroleum ether, diethyl ether, chloroform, ethyl acetate, acetone, ethanol and methanol. Phytochemical for various constituents including was analyzed. Positive results came for alkaloids, amino acids, sterols, terpenes, glycosides and proteins while negative results was for phenols, flavonoids, resins and sugars (Khan *et al*., 2010). In this research, the crude aqueous extract of radhuni seeds was obtained through conventional method. Phytochemical screening was done for alkaloids, terpenoids, tannins, saponins, steroids, phenolic compounds, flavonoids and cardiac glycoside and all tested positive. The result varied because the seeds are not exactly the same and the extract is different as well but as closely similar seeds so comparative results could be taken in consideration.

A handful number of researches have been done with fenugreek seeds. A study was done with aqueous and methanol seed extract of fenugreek where extract was concentrated in a rotary evaporator at 50 °C. In the Muller Hinton agar, agar diffusion method was used to measurement the antibacterial activity on bacterial isolates of *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeuroginosa,*

Escherichia coli, Proteus vulgaris and *Klebsiellapneumoniae.* The extract showed highest activity against *Escherichia coli* while moderate response was showed against *Staphylococcus aureus, Pseudomonas aeuroginosa, Streptococcus pyogeenes* and *Proteus vulgaris* showed lowest response. *Klebsiellapneumoniae* showed negative response (Abdalah, 2011). Another study was conducted with aqueous, ethanol, benzene, chloroform, hexan and petroleum ether extracts of fenugreek and antimicrobial activity was done with agar well diffusion method against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsilla* and showed negative results for all (Nasrulla *et al,* 2010). In this research the ethanolic and the methanolic extract of fenugreek showed negative results for *Pseudomonas aeuroginosa, Escherichia coli. Staphylococcus aureus* and *Klebsiellapneumoniae* which were taken from eleven tested bacteria. Only *B. cereus* gave zone of inhibition for fenugreek extracts in nutrient agar media. Here, the extract was concentrated in water bath at 80 °C.

The antibacterial activity of *T. foenum-graecum* leaves extracts by agar well diffusion method was found maximum on *Serratiamarcescens* with a zone of inhibition of 12.33±0.57 mm by aqueous extract followed by inhibition of *Bacillus cereus* (ZOI = 11.50±0.50 mm) by the methanol extract. (Dharjiya *et al*, 2016) In this study, only *B. cereus* gave highest zone of inhibition of 20.47 mm for the 7th day methanol extract while lowest zone of inhibition of 11.87 mm was for the 5th day ethanol extract within the eleven test microbes where *Pseudomonas aeuroginosa* and *Escherichia coli* also showed negative results. In a study the antibacterial assay of fenugreek seeds ethanol extract was done against *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella* spp. where the result was negative for all (Faraj, 2014). The result is exactly similar to this study as in this study all of these does not give any zone of inhibition. In a study, fenugreek seed's phytochemical assay was carried out for alkaloids, flavonoids, tannins, saponins, starch, essential oil and steroids and the result was positive for all (Swati*et al,* 2014). In another research with fenugreek seed's phytochemical screening was done for alkaloids, tannins, saponins, sterols, phenolic compounds, flavonoids and cardiac glycoside where except tannins all gave positive result (Dharjiya *et al*, 2016). But in this study, phytochemical screening was done for alkaloids, terpenoids, tannins, saponins, sterols, phenolic compounds, flavonoids and cardiac glycosides where it is found that except alkaloids all other phytochemicals are present in fenugreek seed extract. It is actually very difficult to compare directly all of the information of different studies with this research due to a number of factors, such as variability in composition or content of active agents between plants due to origin from various geographical regions, harvesting seasons, growth and drying conditions, or using plant material of diverse maturity. Other factors which manipulate the outcome of antimicrobial testing engross differences in experimental design including inoculums size, growth phase, strain susceptibility, culture medium used, etc. Moreover, powdered seed extracts might have considerable loss of antibacterial activity in comparison to extracts prepared by using crushed seeds, which could be attributed to inactivation of the active antibacterial substances by the heat generated during grinding of the seeds (using an electric blender) (Arora and Kaur, 2009).

In this research comparison between radhuni seed extracts and fenugreek seed extracts had been analyzed. The fact that fenugreek showed antimicrobial activity only against *B. cereus*, the comparing was done only on this pathogen where radhuni extract has the larger zone of inhibition which indicates that radhuni seed has more potential activity than fenugreek seed. Another important fact of the research is the activity index (AI) values of radhuni and fenugreek seeds from different extraction methods. By quantitatively compared to the respective standard antibiotics, AI values are used to discover the prospective of antimicrobial activity of an extract. A research conducted by Dharjiya *et al*, showed AI value of *B. cereus* and *P. aeruginosa* was 0.761 and 0.633 respectively for fenugreek leave extract in methanol. And in this study only *B. cereus* gave zone of inhibition hence AI value of this is highest for the 7th day methanol extract which is 0.76 and lowest for the 5th day methanol extract which is 0.47.For Radhuni seed extract5th day methanol extract of *S. flexineri* is higher than that5th day methanol extract of *B. subtilis* which has highest zone of inhibition. From the study, it is expected that the findings of this study may inspire, motivate and help other researchers to work further and deeper with these household condiments like radhuni and fenugreek. Moreover, these could work as less expensive antimicrobial agents to do clinical trials by alternating the commercial antibiotics with extracts of these two seeds or in amalgamation with different spices' extracts incorporating analogous antibacterial properties which will definitely be an advantage for people of developing countries like Bangladesh.

Conclusion

Today's medical world is countering the two most enormous challenges which are antibiotic toxicity and multi drug resistant pathogens that impinging on directly not only the pharmaceutical and medicine industries but also the food and beverage industries. According to this research, *Carumroxburghianum* undeniably posses excellent potency of antimicrobial activity against diverse pathogenic microorganisms where *Trigonellafoenum-graecum* showed very little influence in antimicrobial activity. The traditional use of radhuni seeds for infectious diseases is promising against various bacteria and disease causing pathogens and for developing new antimicrobial root canal irrigating solutions whereas fenugreek seed is considered as less potential than radhuni.

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REFERENCES

Abdalah, M.E. 2011. The study of Antibacterial activity of fenugreek *(Trigonellafoenum-graecum)* Seeds extract. *Iraq Academic Scientific Journals, 6*(3), 145-155.

Aggarwal, N. K., Dhiman, R., and Kaur, M. 2015. Comparative Evaluation of Antimicrobial Activities of Commonly Used Indian Spices Against Microbes Associated with Juices. *Research Journal of Microbiology, 10*(4), 170-180.

Al-abdeen, S.S.Z., Faraj, B.M. and Nasrulla, O.J 2010.Antibacterial Effects of Fenugreek

(*Trigonellafoenum-graecum*). *Iraq Academic Scientific Journals, 10*(2), 133-140.

Alizadeh, A., Zamani, E., Sharaifi, R., Nikkhah M.J. and Nazari, S. 2010. Antifungal activity of some essential oils against toxigenic *Aspergillus species*. *Communications in Agricultural and Applied Biological Sciences*, 75(4), 761-767.

Basch, E., Ulbricht, C., Kuo, G., Szapary, P. and Smith, M. (2003).Therapeutic applications of fenugreek. *Altern Med Rev.,* 8(1), 20-7.

Bukvicki, D., D. Gottardi, A.K. Tyagi, M. Veljic and P.D. Marin *et al*. 2014.*Scapanianemorea*liverwort extracts: Investigation on volatile compounds, *in vitro* antimicrobial activityand control of *Saccharomyces cerevisiae*in fruit juice. *LWT - Food Science and Technology, 55*(2), 452-458.

Dharjiya, D., Jasanti, H., Khatranti, T., Kapuria, M., Pachchigar, K., and Patel, P. 2016. Evaluation of Antibacterial and Antifungal Activity of Fenugreek (*Trigonellafoenum-graecum*) Extracts. *International Journal of Pharmacy and Pharmaceutical Sciences, 8*(4), 212-217.

Faraj, B. M., Al-Zahawi, A. R., and Osman, R. M. 2014. Antimicrobial assessment of crude extracts of Fenugreek against Endodontic pathogens. *Peak Journal of Medicinal Plant Research, 2* (2),23-26.

Flammang, A., Cifone, M., Erexson, G., and Stankowski, L. 2004. Genotoxicity testing of a fenugreek extract. *Food and Chemical Toxicology, 42*(11), 1769-1775.

Heinrich, M., Barnes, J., Gibbons, S. and Williamson, E. 2003.Families yielding important phytopharmaceuticals in fundamentals of pharmacognosy and phytotherapy. p. 32-33. Edinburgh: Churchill Livingstone.

Iqbal, B., Chaudhry, A.H., Hassan, A., Sahar, A., Akhtar, T. M. and Ali, S. 2014. Physico- chemical investigation and antimicrobial activity studies on essential oil of *Carumroxburghianum. International Journal of Pharmaceutical Chemistry,* 4(4),151-154.

Kalra, E. K. 2003. Nutraceutical-definition and introduction. *American Association of Pharmaceutical Sciences*, *5*(3), 27-28.

Kaur, G. J. and Arora, D. S. 2009. Antibacterial and phytochemical screening of *Anethumgraveolens*, *Foeniculumvulgare* and *Trachyspermumammi*.*BMC Complementary and Alternative Medicine, 9*(1).

Khalsa, Singh, K. P and Tierra, M. 2008."The Way of Ayurvedic Herbs", p. 123.

Khan, A. U., Khan, R., Zakir, M., Afaq, S. H. and Latif, A. 2010. Activity of solvent extracts of*Prosopisspicigera*, *Zingiberofficinale* and *Trachyspermumammi* against multidrug resistant bacterial and fungal strains. *The Journal of Infection in Developing Countries, 4*(05), 292-300.

Khan, M., Khan, A., N. and Gilani, A. 2012.Gut and airways relaxant effects of *Carumroxburghianum*. *Journal of Ethnopharmacology, 141*(3), 938-946.

Khan, R., Islam, B., Akram, M., Shakil, S., Ahmad, A., Ali, S.M., Siddiqui, M., and Khan, A. U. 2009. Antimicrobial activity of five herbal extracts against multi drug resistant (MDR) strains of bacteria and fungus of clinical origin. *Molecules*, *14*(2), 586-597.

Kiessling, C. R., Cutting, J. H., Loftis, M., Kiessling, W. M., Datta, A. R., and Sofos, J. N. 2002. Antimicrobial resistance of food-related *Salmonella* isolates, 1999-2000. *Journal of Food Protection, 65*(4), 603-608.

Minosuke, H. 1958. Umbelliferae of Asia (Excluding Japan). Botanical Institute, Vol. 1, p.182. Japan: Kyoto University, Kyoto.

Nanasombat S., Prasertsin V., Graisin K., Shain H. and Thanaboripat B. 2002.Efficacy of New Enzyme-Linked Immunosorbent Assay for Rapid Detection of Salmonella in Foods. Government Pharmaceutical Organization Report, Bangkok, 51:53-57.

Peerakam, N., Punjaisee, S., Buamongkol, S., Sirisaard, P., Julsrigival, J. and Chansakaow, S. 2014. Chemical compositions, antioxidant and antibacterial activities of essential oils from *Anethumgraveolens* L. and *Trachyspermumroxburghianum* (DC.) Craib grown in Thailand. *Journal of Natural Sciences Research, 12*(4).

Raghuram, T. C., Sharma, R. D., Sivakumar, B., and Sahay, B. K. 1994. Effect of fenugreek seeds on intravenous glucose disposition in non-insulin dependent diabetic patients. *Phytotherapy Research, 8*(2), 83-86.

Shan, B., Cai, Y., Brooks, J. D. and Corke, H. 2007. The in vitro antibacterial activity of dietary spice and medicinal herb extracts. *International Journal of Food Microbiology, 117*(1), 112-119.

Sheikhlar, A. 2013. *Trigonellafoenum-graecum* L. (Fenugreek) as a Medicinal Herb in Animals Growth and Health. *Science International, 1*(6), 194-198.

Sherif, F. M. 2015.Screening of Antimicrobial Activity of Fenugreek Seeds. *Pharmacy & Pharmacology International Journal, 2*(4).

Solomon, K., Mony, M., Zachariah, M.P. and Solomon, J. 2011. Preliminary phytochemical screening of the pericarp of *Crataeva magna* (Lour.) DC. A Medicinal Tree. *Asian Pacific Journal of Tropical Biomedicine,* S129-S130.

Tiran, D. 2003. The use of fenugreek for breast feeding women. *Complementary Therapies in Nursing and Midwifery, 9*(3), 155-156.

Appendix

1. 10% Ferric chloride

   1g of Ferric chloride in 10 ml distilled water

2. 5% Ferric chloride

   1g of Ferric chloride in 20 ml distilled water

3. 20% sodium hydroxide solution

   2g of sodium hydroxide in 10 ml distilled water

4. Glacial acid

   Acetic acid

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