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Influence of Growth and Ripening of *Physalis minima* L. Fruit on its Antibacterial Potential

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ABSTRACT

Plants have been exploited to a large extent for the treatment of human diseases in different parts of the world. Plant products with antibacterial properties have obtained enormous emphasis for exploration of its novel bioactive compounds. Underutilized edible fruits of *Physalis minima* L. have been screened at their successive stage of growth and ripening to identify its antibacterial potential using agar well diffusion method. Various non-polar to polar infusion extracts were used to determine zone of inhibition and minimum inhibitory concentration against medically important bacterial strains namely *Bacillus cereus*, *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella paratyphi* and *Salmonella typhi*. Methanol and ethyl acetate extracts of mature and ripened fruit showed significant activity against *Micrococcus luteus* and *Bacillus subtilis*. The study also demonstrates the influence of maturity indices of *P. minima* fruit on its antibacterial potential and demands further studies to identify the bioactive natural compounds present so as to serve and facilitate pharmacological studies.

Key words: Antibacterial, growth and ripening, maturity indices, *Physalis minima* L., underutilized fruits

INTRODUCTION

Since the dawn of human civilization plants have made large contributions to facilitate human health and well being (Singh *et al*., 2012). They are rich sources of herbal medicines and possesses invaluable, incredible source for curing various diseases (Archana *et al*., 2011). But today the difficulties have become more acute due to the overdependence of humans on fewer plant species. Medicinal potentials of most common plants have been extensively studied and compiled but the lack of information regarding the potential of some less commonly used plants limits the use of these underutilized plants. Throughout the world numerous researchers have emphasized on the diversity and use value of these wild edible plants (Maikhuri *et al*., 2000; Dhyani *et al*., 2007; Scherrer *et al*., 2005; Pieroni *et al*., 2007) and perceived that the nutritional and medicinal values of many wild plants are higher than the commonly used fruits and vegetables (Patel and Rao, 2009; Patel and Rao, 2011; Namrata *et al*., 2011).

During the process of growth and development of fruit, it undergoes through a series of developmental transitions, involving coordinated changes in a number of catabolic and anabolic reactions (Duhan *et al*., 1992) which leads to the synthesis or degradation of wide range of bioactive compounds. Hence, fruits at varying maturity levels may possess vivid bioactive compounds, which need to be studied so as to provide maturity indices for its usage as a source of food or medicine. It has been proven that ethno-botanically derived compounds have greater potential bioactive compounds than that derived from random screening and therefore they provide a greater potential for product development (Chanda *et al.*, 2011).

According to World Health Organization (WHO), infectious diseases are the number one cause of deaths world wide. The emergence of antibiotic-resistant microorganisms had swiftly reversed the advances of previous fifty years of research on antibiotics (Menghani *et al.*, 2011). Furthermore, some antibiotics have serious undesirable side effects which limit their applications (Shan *et al.*, 2007). Hence, the ultimate goal of the leading drug companies and the academia is to hunt for novel therapeutic/antibacterial agents that are effective with minimal side effects (Sati *et al.*, 2011). Since a diverse range of bioactive compounds that offer potentials for the treatment of chronic and infectious diseases can be found most especially in traditional medicinal plants (Ynalvez *et al.*, 2012). Present approaches have been made to develop new antibacterial compounds to treat various diseases using probiotics, prebiotics, organic acids and medicinal plants (Thirunavakkarasu *et al.*, 2011).

Physalis minima L. of family Solanaceae consists of about 100 species are found to be distributed world wide. The plant is an annual herb, quick growing and high fruit yielding (Patel *et al.*, 2011). The fruits are enclosed in an inflated, bladder like calyx or husk, juicy and possess numerous seeds within. The ripe fruits are edible and used as an appetizer, diuretic, laxative and tonic (Chopra *et al.*, 1986; Patel *et al.*, 2011). The extracts from the leaves have also been reported to show anticancer activity (Duke and Ayensu, 1985). Therefore, looking into the potential of this underutilized plant, the present study have been undertaken to investigate the influence of various maturity levels of *Physalis minima* fruit on its antibacterial potential.

MATERIALS AND METHODS

The fresh underutilized fruit samples of *Physalis minima* L. were collected from the vicinity of Vallabh Vidyanagar, Gujarat, India at their sequential stages of growth and ripening. The fruit samples were dried at room temperature, grounded to powder and stored in air tight containers until further use. The infusion extraction method (Houghton and Raman, 1998) was used for which non polar solvent series starting from diethyl ether, ethyl acetate, acetone, methanol and water were used. The resulting extracts were concentrated by drying them at room temperature and finally stored in refrigerator (4°C) until further use.

The following 4 gram positive and 4 gram negative bacterial cultures were used in this study namely MTCC-430 *Bacillus cereus* (BC), MTCC-121 *Bacillus subtilis* (BS), MTCC-106 *Micrococcus luteus* (ML), MTCC-435 *Staphylococcus epidermidis* (SE) and MTCC-443 *Escherichia coli* (EC), MTCC-109 *Klebsiella pneumoniae* (KP), MTCC-735 *Salmonella paratyphi* (SP), MTCC-734 *Salmonella typhi* (ST) respectively. All the bacterial pure cultures obtained from MTCC (Microbial type culture collection, Chandigarh, India) were used for the present study.

The antibacterial activities of the infusion extracts were screened using agar well diffusion method (Perez *et al.*, 1990). All the bacterial cultures used were grown on nutrient agar medium (pH 7.4) at 37°C. A 0.5 Mc Farland turbidity standard was used to measure the density of bacterial cells (Ogbonnia *et al.*, 2008). Antibiotics such as ciprofloxacin and doxycycline (20 $\mu g\ mL^{-1}$) were used as positive controls, while 100 and 50% DMSO were used as negative controls. The antibacterial activities were determined by measuring the diameter (mm) of the inhibitory zone. All the bioassays were carried out in triplicate to minimize the error.

The Minimum Inhibitory Concentration (MIC) of the samples, which resulted in an inhibition zone of 10 mm or more, was determined. MIC values have been evaluated using serial broth dilution method ranging from 8 to 0.250 mg mL^{-1}. Finally the presence of live bacterial population was determined using 2, 3, 5-triphenly tetrazolium chloride test (Patel, 2009). The solutions containing DMSO and nutrient broth were used as controls. The MIC values of the samples were carried out in three replicates to confirm the activity.

RESULTS

Various infusion extracts of *Physalis minima* were screened to understand its antibacterial potential against some selected bacterial strains. Methanol extracts proved to be the best extract exhibiting higher zones of inhibition, followed by acetone, diethyl ether, ethyl acetate and water. The methanol extract of mature fruit exhibited good to better activity against *Micrococcus luteus* (18 mm) followed by *Bacillus cereus* (13 mm), *Escherichia coli* (11 mm), *Salmonella paratyphi* (11 mm) and *Salmonella typhi* (10 mm). Also methanol extract of young and ripened fruit was effective in regulating the growth of *Escherichia coli* with 10 and 11 mm inhibition zone respectively, while pre-mature fruit extracts were effective against *Micrococcus luteus* (15 mm). However, using acetone extracts of young and pre-mature fruit showed moderate to no activity against all the bacterial strains used but extracts of mature fruit inhibited the growth of *Staphylococcus epidermidis* (13 mm). In contrast, acetone extracts of pre-ripened and ripened fruit exhibited high inhibition zone against *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus epidermidis* and *Escherichia coli*, while other bacterial strains were least affected. Diethyl ether extracts mainly affected the growth of *Bacillus cereus*, *Bacillus subtilis* and *Micrococcus luteus*. Similarly, ethyl acetate extracts of mature fruit also inhibited the growth of *Bacillus subtilis* and *Micrococcus luteus* to 10 mm, while ripened fruit exhibited an inhibition zone of 16 mm against *Bacillus subtilis*. In contrast, water extract exhibited less or no activity against all the bacterial strain used. *Bacillus cereus* and *Bacillus subtilis* both were found to be highly resistant and exhibited no activity using water extracts (Table 1).

The MIC values of 8 mg mL^{-1} and more than 8 mg mL^{-1} were obtained using diethyl ether and methanol extracts of mature fruit against *Bacillus cereus* and *Escherichia coli*, respectively, while methanol extract of pre-mature fruit exhibited lower MIC value (2 mg mL^{-1}) against *Micrococcus luteus*. However, among the mature fruit extracts diethyl ether extract resulted a MIC value of more than 8 mg mL^{-1} against *Micrococcus luteus*, ethyl acetate extract was also effective at 4 mg mL^{-1} against *Micrococcus luteus* and *Bacillus subtilis*. Acetone extract of mature fruit inhibited the growth of *Staphylococcus epidermidis* at 4 mg mL^{-1} and methanol extract against *Bacillus cereus* at 1 mg mL^{-1}, *Micrococcus luteus* at 0.25 mg mL^{-1}, while more than 8 mg mL^{-1} inhibited the growth of *Escherichia coli*, *Salmonella paratyphi* and *Salmonella typhi*. Moreover, the pre-ripened fruit inhibited the growth of *Bacillus cereus* at 4 mg mL^{-1} and *Micrococcus luteus* at 8 mg mL^{-1} using diethyl ether extract, while acetone extract regulated the growth of *Bacillus cereus* at 4 mg mL^{-1} but more than 8 mg mL^{-1} inhibited the growth of *Bacillus subtilis*. Lastly, the ripened fruit inhibited the growth of *Bacillus subtilis* (1 mg mL^{-1}) using diethyl ether extract and ethyl acetate extract against *Bacillus subtilis* (0.5 mg mL^{-1}), acetone extract against *Bacillus subtilis* and *Staphylococcus epidermidis* each at 4 mg mL^{-1} and *Escherichia coli* with more than 8 mg mL^{-1}, while methanol extract inhibited the growth of *Escherichia coli* at 4 mg mL^{-1} (Table 2).

Table 1: Zone of inhibitions of *Physalis minima* L. fruit at its sequential stages of growth and ripening

		Zone of inhibition (mm)							
		Gram +ve bacteria				Gram -ve bacteria			
Infusion extracts	Stages of fruit development	BC	BS	ML	SE	EC	KP	SP	ST
Di-ethyl ether	1	10	1	7	4	-	7	-	3
	2	9	2	5	8	-	4	-	4
	3	7	4	10	2	-	7	-	8
	4	12	8	11	-	5	-	2	4
	5	8	15	7	-	3	-	-	6
Ethyl acetate	1	3	7	7	5	1	-	1	8
	2	4	7	8	7	5	-	-	8
	3	4	10	10	-	-	1	2	5
	4	7	9	5	5	-	7	4	4
	5	5	16	7	7	-	5	2	3
Acetone	1	-	7	-	8	-	7	-	-
	2	-	5	-	5	-	6	-	-
	3	-	9	-	13	-	5	-	-
	4	13	10	5	8	4	-	5	2
	5	-	12	4	11	11	-	4	4
Methanol	1	2	2	2	1	10	2	2	2
	2	7	5	15	4	7	3	7	7
	3	13	8	18	8	11	-	11	10
	4	6	2	-	5	8	-	2	8
	5	5	7	-	7	11	-	5	9
Water	1	-	-	-	1	5	-	-	1
	2	-	-	-	2	3	-	-	2
	3	-	-	9	5	-	-	-	3
	4	-	-	-	-	-	2	-	7
	5	-	-	-	-	-	5	-	4

BC: *Bacillus cereus*, BS: *Bacillus subtilis*, EC: *Escherichia coli*, KP: *Klebsiella pneumoniae*, ML: *Micrococcus luteus*, SE: *Staphylococcus epidermidis*, SP: *Salmonella paratyphi*, ST: *Salmonella typhi*

Table 2: Minimum inhibitory concentration values of *Physalis minima* L. fruit extracts against some selected bacterial strains

Stage of development	Extracts	Bacterial strains	MIC (mg mL^{-1})
Young	Di-ethyl ether	BC	8
Pre-mature	Methanol	ML	2
Mature	Ethyl acetate	ML	4
		BS	4
	Acetone	SE	4
	Methanol	BC	1
		ML	0.25
Pre-ripened	Di-ethyl ether	BC	4
		ML	8
	Acetone	BC	4
Ripened	Di-ethyl ether	BS	1
	Ethyl acetate	BS	0.5
	Acetone	BS	4
		SE	4
	Methanol	EC	4

BC: *Bacillus cereus*, BS: *Bacillus subtilis*, EC: *Escherichia coli*, ML: *Micrococcus luteus*, SE: *Staphylococcus epidermidis*

DISCUSSION

The infusion extracts of *Physalis minima* were found to have good activity against all organisms tested, except the water extracts all other extracts were found to have more or less some zone of inhibition against all bacterial strains tested. Fruits at mature and ripened stages have shown its prominent potential in effectively inhibiting the growth of five bacterial strains. The effect of the various solvents used for the preparation of infusion extracts was clearly observed in the present study and methanol extracts resulted to possess higher antibacterial potential. The results of the study are in view of the observations made by Sundaram *et al.* (2011) and Sonibare *et al.* (2011). The beneficial medicinal effects of plants usually results due to the combination of secondary metabolites like phenols, flavanoids, steroids, fatty acids, alkaloids, tannins, resins, gums etc which provide some physiological action (Mishra and Mishra, 2011). However the difference observed in various infusion extracts may be due to strain variability in the sensitivity and/or in the tests (Al-Zoreky, 2009). Lack of activity in some extracts according to Farnsworth (1993) can thus only be proven by using large doses or the active principle might be present in high enough quantities and there could be other constituents exerting antagonistic effect or negating the positive effects of the bioactive agents (Jager *et al.*, 1996). Less or no activity in other solvents has also been regarded as it may be due to the degradation of active compounds during extraction, lack of solubility etc. (Premanath *et al.*, 2011).

The present study also suggests that the gram positive bacterial strains are less resistant than the gram negative bacterial strains. The results obtained are in accordance with the results of Yaghoubi *et al.* (2007) and Zongo *et al.* (2010) who observed gram negative bacterial strains to be more resistant than that of gram positive bacterial strains. Ahmad and Beg (2001) have also opined that the gram-positive bacteria are considered to be more sensitive as compared to gram-negative because of the differences in their cell wall structures (Balasundaram *et al.*, 2011). Many medicinal plants have been reported to exhibit antimicrobial activity by cell membrane lyses, inhibition of protein synthesis, proteolysis enzymes and microbial adhesions (Agbafor *et al.*, 2011). Besides, Roychoudhury (1980) is of the opinion that the extracts of *Physalis minima* varies in its degree of inhibition against tobacco mosaic virus. Moreover, it has been strongly believed that crude extracts from pants are more effective than isolated components due to their synergistic effect (Jana and Shekhawat, 2010). Besides as fruits have been the main subject for researchers to be investigated since their bioactive compounds close related with herbs, commonly referred as phytochemicals such as anthocyanins, alkaloids, carotenoids, flavonoids, polyphenols and tannins which are present in the fruits and vegetables are known to be effective as antibacterial substances against a wide array of infectious agents (Jamine *et al.*, 2007; Prasad *et al.*, 2008) and hence are gaining lot of interest due to their functional property (Li *et al.*, 2006; Rao and Rao, 2007).

CONCLUSIONS

The present study exhibits prominent inhibitory effect using methanol and ethyl acetate extracts of *Physalis minima* fruit at mature and ripened stage. The study provides new insight to identify the bioactive compounds and demands scientific evidence based validation of bioactive phytochemicals, since medicinal plants are regarded as the sleeping giants of pharmaceutical industries.

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