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Phytochemical analysis, antioxidant and antimicrobial activity of *Raphanus sativus* and *Citrus Limon Peel*

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Abstract
Radish (*Raphanus sativus*) and *Citrus limon* is consumed in India and considered as one of the major source of human diet. The major objective is to determine its antimicrobial and antioxidant activity of Radish (*Raphanus sativus*) especially its leaves and *Citrus limon* i.e. peel. In this study, phytochemical studies were conducted in order to analyze the presence of secondary metabolites and determined its antimicrobial (using three bacterial strains) and antioxidant (using DPPH, 2,2-diphenyl-1-picryl-hydrazyl-hydrate assay) activity. The results of these studies showed the existence of metabolites in different extracts and also showed its antimicrobial and antioxidant activity as well.

Keywords: *Raphanus sativus*, *Citrus limon*, antimicrobial, antioxidant

Introduction
In our day to day to day life, intake of several fruits and vegetables plays an important role in improving human health and prevention of several diseases. These are considered them as one of the rich source of several vitamins (C, A, B_6, thiamine, niacin, E) including minerals and dietary fibers. These constituents may played an important role in boosting our immune system [1, 2]. In addition, it may also prevent certain chronic diseases or disorders i.e. cardiovascular diseases, cataract and cancer [1-3]. As per the literature, fresh fruits and vegetables containing rich sources of micronutrients along with macronutrients and showed antioxidant property as well. In general, micronutrients are present in the form of minerals (like iron and calcium) and vitamins (like vitamin C, folic acid, B complex vitamins and cartenoids) whereas, macronutrients are present in the form of complex carbohydrate/fibre. It has also been reported that fruits and vegetables also shows the presence of many anticarcinogenic secondary metabolites like glucosinolates, organosulphur, carotenoids etc. [4-6].
Radish (*Raphanus sativus* L.), biennial herbaceous plant, belonging to the family *Brassicaceae*; grown for its young tender tuberous roots, developed from both primary root and hypocotyls known for its commercial and nutritional values [7-10]. It has been previously reported that radish is a good source of vitamin C (ascorbic acid) and minerals like calcium, potassium and phosphorus. As per literature, different parts of the radish plant show varying degrees of biological activities. Leaves and roots of *Raphanus sativus* have been used to treat cancer and said to be useful in urinary complaints, piles and in gastrodynia while seeds and aerial parts of *Raphanus sativus* contains raphanin compound, responsible for its strong antifungal and antibacterial activity [8-10]. Similarly, citrus genus belongs to the large family *Rutaceae*, were found to contain flavonoids, coumarins and cartenoids, which play important role in prevention of many degenerative diseases such as Alzheimer's and Parkinson's disease [11, 12]. As per literature, citrus fruits like lemon, orange, grapefruit, limes, were found to excellent source of vitamin C, folic acid, carotenoids, dietary fibres, potassium, selenium and a wide range of phytochemicals. Vitamin C and citrus flavonoids possess antioxidant properties [12, 13]. They possess excellent hydroxyl radical scavenging activity superoxide scavenging activity and antilipoperoxidant activity. As per latest research, citrus flavonoids possess anticarcinogenic and antitumour activities [11-14]. The objective of our study is to screen the antimicrobial and antioxidant property of aqueous extract of *Raphanus sativus* leaves and *Citrus limon peel*.

Material and Methods
Collection of Plant material
The collection of fresh and healthy plant material were done from the local market. Leaves of *Raphanus sativus* were then washed thoroughly under tap water and dried under shade, until all the water content was lost completely. Dried plants were then crushed and powdered using electric blender. The fine particles were then separated with the help of a sieve and were stored in an air tight container until further use. Similarly, *Citrus limon* were then washed thoroughly under tap water followed by removal of a peel. The peels of citrus fruits were then crushed and powdered using electric blender. The fine particles were then separated with the help of a sieve and were stored in an air tight container until further use.

Preparation of Plant extracts
The extraction of the *Raphanus sativus* leaves was done with different solvents, varying in polarity. 200 grams of the air dried sample were weighed and extracted with 3 different solvents; i.e. Petroleum ether, Chloroform and aqueous (using PBS, phosphate buffered saline). The extraction was done by a cold maceration process. 20 grams of *Raphanus sativus* leaves powder was mixed with 100 ml of solvent extracts in a conical flask. The conical flasks was kept with intermittent shaking for 72 h with regular agitation under controlled environmental conditions. The extracts so

obtained were then filtered by using muslin cloth and Whatman's (No.1) filter paper The filtrate were concentrated using an IKA rotary evaporator at a temperature not exceeding the boiling point of the solvents. The residues were then stored below ambient temperature in a plastic screw cap tube, until further use. The extraction of the *Citrus limon peel* was done with different solvents, varying in polarity. 200 grams of the air dried sample were weighed and extracted with 3 different solvents; i.e. Petroleum ether, Chloroform and aqueous (using PBS). The extraction was done by a cold maceration process. 20 grams of *Citrus limon peel* powder was mixed with 100 ml of solvent extracts in a conical flask. The conical flasks were kept with intermittent shaking for 72 hours with regular agitation under controlled environmental conditions. The extracts so obtained were then filtered by using muslin cloth and Whatman's (No.1) filter paper The filtrate were concentrated using an IKA rotary evaporator at a temperature not exceeding the boiling point of the solvents. The residues were then stored below ambient temperature in a plastic screw cap tube, until further use.

Preliminary phytochemical Screening

For phytochemical screening, the plant material was washed thoroughly, dried under the open shade, ground and stored in paper bags at room temperature. These plant samples were screened to detect different phytochemicals. 2 ml of each extract was measured into a test tube for each of the tests and concentrated by evaporating the extractant in a trough. Tests were carried out for carbohydrates, reducing sugars, tannins, phenols, flavonoids, alkaloids, steroids, glycosides and triterpenoids [15].

Culture Media and strains

To evaluate the potential antimicrobial activity of *Raphanus sativus* and *citrus limon* peel, using three bacterial strains were selected namely *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Salmonella enteritidis.* The Nutrient agar medium (NAM) was used to culture microbes required for antimicrobial susceptibility test. Microbial strains were cultured on to the agar plate and the broth, followed by overnight incubation at 37° C. Selected bacterial strains mentioned above were suspended in normal saline and inoculated in Mueller Hinton agar.

Antimicrobial activity using disc diffusion method

The antimicrobial activity of the *Raphanus sativus* leaves extracts and *Citrus limon peel* were tested using disc diffusion method. A 100 µl of microbial culture of the respective strains is to be spread with the help of an L-shaped spreader containing MHA (20 ml/ plate). The sterile discs (6 mm in diameter) containing residues of the extracts were independently impregnated on the agar plates which have been previously been inoculated with the selected microbial strain. Erythromycin was used as a positive control for bacteria. Discs without samples were used as a negative control. The plates were then incubated at 37° C for 24 hours. Antimicrobial activity was then determined by measuring the diameter of the growth - inhibition zone in millimeters [16].

Antioxidant activity using DPPH free radical assay

Antioxidant activity was determined in *Raphanus sativus* leaves extracts and *Citrus limon peel* using DPPH free radical assay. In this study, test sample in the form of extracts were reacted with DPPH radical dissolved in an ethanol solution [17]. This reaction mixture is mainly compromised of extract (0.25 ml), absolute ethanol (1.5 ml) and DPPH radical solution (0.15 ml dissolved in 0.25 M Methanol). In other word, DPPH reacts with one of the antioxidant compound present in extract, which can donate or release hydrogen; it is reduced. So, color will change from deep violet to light yellow and its absorbance were read at 517 nm after 100 min of reaction using a UV-VIS spectrophotometer. For these studies, ethanol (1.625 ml) and sample (0.25ml) selected as blank, whereas the control solution contained ethanol (1.75 ml) and DPPH radical (0.15 ml) solution. The scavenging activity percentage (AA %) was determined by using this equation i.e.

Antioxidant activity=100-[(Sample absorbance- Blank absorbance)*100/absorbance control]

Statistical analysis

The difference between control and variable concentration of glucan is determined through Bonferroni multiple comparison test (One way ANOVA).

Results and Discussion

In Indian folk medicine, *Raphanus sativus* and citrus fruits are mainly used as a household remedy especially for the treatment or recovery for many diseases. The most familiar examples are jaundice, liver diseases, indigestion and other gastric pains. In literature, *Raphanus sativus* and *Citrus limon peel* reported unique type of bioactive compounds and is mainly recognized as potential health benefits with respect to humans. In this study, we worked on extracts (leaves of *Raphanus sativus* and peel of *Citrus limon*) using different solvent system for determining its antimicrobial and antioxidant activity. The results of phytochemicals present in crude petroleum ether, aqueous and chloroform extract of *Raphanus sativus* are given in Table.1A As shown in table Amino acids & proteins, flavonoids, glycosides, phenols, saponins are absent in petroleum ether extract. Aqueous extract shows the presence of alkaloids, flavonoids, tannins, phenols and absence of carbohydrates, saponins, amino acid and proteins. Chloroform extract gave positive results for alkaloids, amino acids & proteins, flavonoids, glycosides, tannins and phenols. Similarly, results of phytochemicals present in crude petroleum ether, aqueous and chloroform extract of *citrus fruits* are given in Table.1B As shown in table amino acids and proteins, flavonoids, glycosides, tannins, saponins are absent in petroleum ether extract. Aqueous extract shows the presence of alkaloids, flavonoids, glycosides, carbohydrates, phenols, tannins and amino acid and proteins and absence of saponins. Chloroform extract gave positive results for alkaloids, carbohydrates, flavonoids, glycosides and phenols. In literature, antioxidant effect were measured as per the capability of these extracts using different solvent system to scavenge hydroxyl radicals. The results showed that aqueous extracts (*Raphanus sativus* and *Citrus limon peel*)

showed higher antioxidant properties as compared to petroleum and chloroform extract. This activity could be due to existence of plant secondary metabolites and considered to be naturally occurring antioxidant and antibacterial agents. In literature, reported several phytochemicals are reported in plant and fruit components especially seed, bark, stem, leaf, fruit, peel etc. These components may contained diverse type of natural antioxidants and considered to be one of the most potential source of bioactive phytochemicals. This finding was more consistent related to aqueous extract of *Raphanus sativus* and *Citrus limon peel* indicated better activity in DPPH radical scavenging as compared to petroleum ether and chloroform extract (Fig.1). These studies may showed some variations in the results especially using different solvent system obtained from *Raphanus sativus* and *citrus limon peel*. So, there is variation reported in our studies and it may be happen due to some ecological factors and variations in the chemical composition including some differences using different extraction solvents and existence of various phytochemicals that are present. In addition, environmental factors are also one of the major factor which mainly affect the quantity and quality of bioactive compounds. In antimicrobial activity, chloroform extract of the *Raphanus sativus* had high inhibition zone on *Salmonella enteritidis* i.e. 20 mm (Table 2). The maximum zone of inhibition in case of chloroform extract against *Salmonella enteritidis* i.e. 20 mm, followed by *Pseudomonas aeruginosa* i.e. 15 mm and *Bacillus subtilis* i.e. 14 mm, while minimum or no zone of inhibition found in petroleum ether extract on *Bacillus subtilis and Salmonella enteritidis* whose zone of inhibition was not greater than 0-3 (Fig.2). Aqueous extract of *Raphanus sativus* showed a slight inhibition on *Salmonella enteritidis* i.e. 6mm. Similarly, chloroform extract of the *Citrus limon peel* was found to show inhibition zone on all the bacterial strains i.e. *Bacillus subtilis* that is 6mm, *Pseudomonas aeruginosa* i.e. 8 mm and *Salmonella enteritidis* i.e. 7mm (Table 2). The Petroleum extract and aqueous extract of *Citrus limon peel* was found to show minimum or zone of inhibition (Fig.2). Overall, these plant components may showed antimicrobial activity. Further study, however, needs to be done to identify and characterize the active compounds responsible for the observed properties. Thus, it could be concluded that the extracts of these plant components possessed imperative pharmacological properties, which will be potential to develop natural compounds based pharmaceutical products

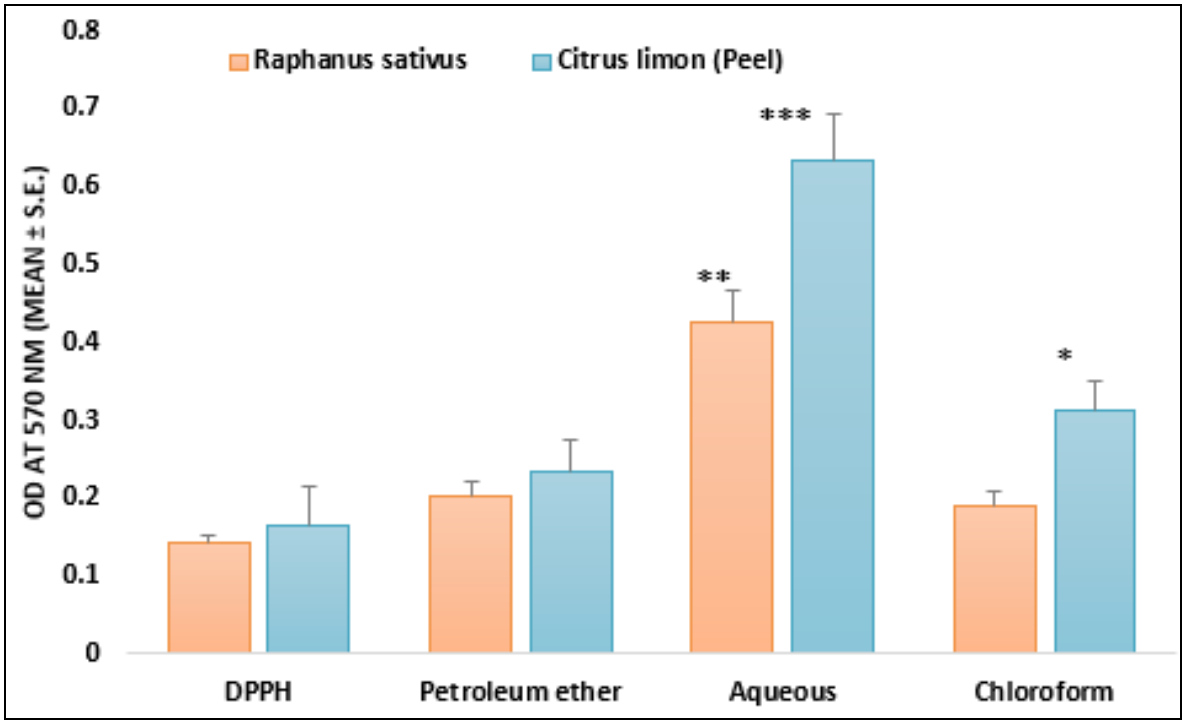


Fig 1: Estimation of antioxidant activity in *Raphanus sativus* and *Citrus limon peel* through DPPH assay. For these studies, ethanol and sample selected as blank whereas control solution contained ethanol and DPPH radical solution. The scavenging activity percentage (AA %) was determined by using this equation i.e. Antioxidant activity = 100 – [(Sample absorbance – Blank absorbance) x100/absorbance control]

Table 1 (A): Phytochemical analysis *Raphanus sativus*

Phytochemicals	Petroleum ether	Aqueous	Chloroform
Alkaloids	+ ve	+ ve	+ ve
Amino acids and proteins	- ve	- ve	+ ve
Carbohydrates	+ ve	- ve	- ve
Flavonoids	- ve	+ ve	+ ve
Glycosides	- ve	- ve	+ ve
Tannins	+ ve	+ ve	+ ve
Phenols	- ve	+ ve	+ ve
Saponins	-ve	-ve	-ve

* -ve: Negative, +ve: Positive

Table (B): *Citrus limon (Peel)*

Phytochemicals	Petroleum ether	Aqueous	Chloroform
Alkaloids	+ ve	+ ve	+ ve
Amino acids and proteins	- ve	+ ve	- ve
Carbohydrates	+ ve	+ ve	+ ve
Flavonoids	- ve	+ ve	+ve
Glycosides	- ve	+ ve	+ ve
Tannins	- ve	-ve	- ve
Phenols	+ve	+ ve	+ ve
Saponins	-ve	- ve	-ve

* -ve: Negative, +ve: Positive

Table 2 (A): Evaluation of antimicrobial activity (A) *Raphanus sativus*

S. No.	Test organism	Inhibition zone (mm)				
		Positive control (Erythromycin)	Negative control	Petroleumether	Chloroform	Aqueous
1	Bacillus subtilis	22	NI	NI	14	NI
2	Salmonella enteritidis	25	NI	NI	20	6
3	Pseudomonas aeruginosa	23	NI	NI	15	NI

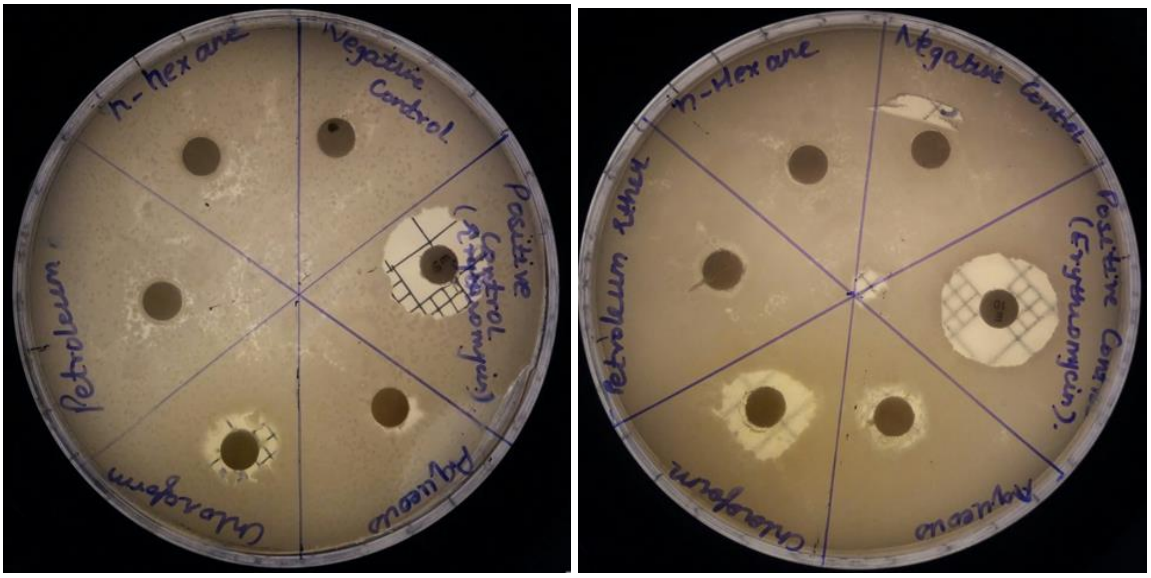
(*NI= NO Inhibition)

Table 2(B): *Citrus limon (Peel)*

S. No.	Test organism	Inhibition zone (mm)				
		Positive control (Erythromycin)	Negative control	Petroleum ether	Chloroform	Aqueous
1	*Bacillus subtilis*	22	NI	NI	6	NI
2	*Salmonella enteritidis*	25	NI	NI	7	NI
3	*Pseudomonas aeruginosa*	23	NI	NI	8	NI

(*NI= NO Inhibition)

A. Raphanus sativus



Pseudomonas aeruginosa Salmonella enteritidis

B. Citrus limon peel

Pseudomonas aeruginosa Bacillus subtilis

Fig 2: Showing antibacterial activity of different extracts of *Raphanus sativus* and *Citrus limon* peel against *Pseudomonas aeruginosa* and *Bacillus subtilis*

Conclusion

This study showed that *Raphanus sativus* and *Citrus limon peel* have different antimicrobial and antioxidant activities. These activities are mainly used and more helpful pertaining to treat several type of diseases including microbial infections. In view of this, plant components may be exploited and used in traditional remedies and possess various beneficial biological activities i.e. antioxidant or antimicrobial activities. Further characterization of these plant components are being conducted through pharmaceutical and biomedical corporations and used them into traditional remedies. Therefore, this study supports the use of these extracts in traditional herbal medicine applications

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