

Evaluation of Antimicrobial Potential and Phytochemical Assessment of *Citrus maxima* Burm. Seeds Extracts Against Respiratory Tract Pathogens

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Abstract- The antimicrobial activity and phytochemical investigation of seeds extracts of *Citrus maxima* Burm. was examined against common respiratory tract pathogens. The pathogens used in this study were *Streptococcus pneumoniae* (MTCC 655), *Staphylococcus aureus* (MTCC 1144), *Pseudomonas aeruginosa* (MTCC 2474), *Streptococcus pyogenes* (MTCC 442), *Haemophilus influenzae* (MTCC 3826) and two fungal pathogens *Candida albicans* (MTCC 227), *Aspergillus niger* (MTCC 921). The plant material was extracted with four different solvents i.e., petroleum ether (PET), acetone (ACE), methanol (MeOH) and water (H₂O) with increasing polarity by Soxhlet apparatus and removed the solvent using vacuum evaporator at 30°C. Antibacterial and antifungal activities were examined by Agar well diffusion method and poisoned food technique, respectively. The methanol (MeOH) extract was found most active as compared to other extracts. The maximum inhibition zone was found against *Staphylococcus aureus* (24±0.88 mm) and minimum against *Candida albicans* (7.66±0.32 mm). Minimum inhibitory concentrations (MICs) were observed for MeOH extract between 3.12 to 25 mg/mL against *Staphylococcus aureus* and *Candida albicans* respectively. Phytochemical analysis of plant extracts showed the occurrence of alkaloids, saponins, steroids, flavonoids, tannins, resins and phenolic compounds. The antimicrobial activity of the crude extracts of plant represents a considerable outcome for the treatment of respiratory diseases. [Ajeet Singh and Navneet. **Evaluation of Antimicrobial Potential and Phytochemical Assessment of *Citrus maxima* Burm. Seeds Extracts Against Respiratory Tract Pathogens.** *N Y Sci J* 2016;9(9):4-10]. ISSN 1554-0200 (print); ISSN 2375-723X (online). <http://www.sciencepub.net/newyork>. 2. doi: [10.7537/marsnys090916.02](https://doi.org/10.7537/marsnys090916.02).

Keywords: Respiratory Tract Pathogens, Antimicrobial, Phytochemical, Minimum Inhibitory Concentration, *Citrus maxima*.

Introduction

Traditional plants derived medicines have been extensively used in most parts of the world. The use of the traditional plants for combating microbial disease is becoming the focus of numerous studies [Bhavnani and Ballow, 2000; Chariandy et al., 1999]. Plants have unlimited capacity to synthesize secondary metabolites like tannins, terpenoids, alkaloids, flavo- glycosides and phenols which having antimicrobial properties [Cowan, 1999; Sher, 2009; Das, 2010; Kumar et al., 2015]. Plant derived substances have recently become of great attention owing to their resourceful applications. It has been estimated that 14- 28 % of higher plant species are used in medicinal purposes and that 74 % of pharmacologically active phytochemicals were discovered after following up on ethno medicinal use of the plants [Borah et al, 2012]. In the last couple of decades, it is evident that there is a new development in the research and promotion of plants based drugs. The interest of the peoples has become increasingly towards the herbal medicines [Bisset, 1994; Tyler, 1997; Singh, et al., 2016]. WHO advised to promote the use of phytochemicals. It has been seen that the conventional medicine is increasingly resistive to antimicrobials and other drugs from plants. As the goal to the control of MDR

(Multidrug resistance) bacteria is persuasively tough, phytochemicals are to be used with adapt as antimicrobials. Recently MDR bacteria isolated from clinical samples were used in parallel work of monitoring of antimicrobial activities of crude phytochemicals [Rath and Padhy, 2012; Dubey and Padhy, 2013].

Respiratory tract infections are the most common ailment including allergies, asthma and chronic obstructive pulmonary disease (COPD). The proportion of non-communicable disease deaths in 2008 due to respiratory diseases were 3.9%, with 4.2 million deaths occurred due to asthma and COPD worldwide [WHO, 2010]. The climatic conditions are very complimentary for spread of such diseases commonly transmitted by coughing and sneezing (airborne disease). Some common causal agents are *Escherichia coli*, *Klebsiella pneumoniae* responsible for nosocomial infections [Saonuam et al., 2008], *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Moraxella catarrhalis* for community acquired infections, *Enterobacter cloacae* and *Bacillus subtilis* which cause occupational asthma, respectively [Kayser et al., 1990; Chan-Yenug and Lam, 1986].

Citrus maxima (syn *C. grandis*) commonly known as pummelo, shaddock or chakotra is a perennial tree and edible fruit belonging to family Rutaceae. The fruit is always round shape, big size, a native plant of Asia and commercially grown in India. In traditional medicine, the fruit peel has been widely used for cough, swelling, and epilepsy, because of the efficiency of the volatile oil [Morton, 1987, Scora, 1975]. The hot leaf decoction is used on swellings and ulcers. The fruit juice is taken as a febrifuge. The seeds are employed to cure coughs, dyspepsia and lumbago. The fruit include treatment of coughs, fevers, cardiotoxic, cancer and gastrointestinal disorders [Barrion et al, 2013]. *C. maxima* have been recommended in traditional herbal medicine as the source of diabetic medication or remedy for diabetes. *Citrus* fruits contain flavonoids and limonoids which are proven to possess anti-inflammatory and antitumor activities. The leaves of *C. maxima* contain antihyperglycemic and anti-oxidant property. Fruit juice of *C. maxima* possess hypoglycemic and hypocholesterolemic activities. It has been used as a folk medicine in many countries as antioxidant antibacterial, antifungal, larvicidal, hepatoprotective, anticancer, antiplatelet, antidiabetic, anti-inflammatory [Barrion et al; 2013; Kundusen et al; 2011; Jadhav et al., 2013]. *C. maxima* fruits are very rich source of flavonoids like hesperidin and naringin [Manthey, et al, 1996]. The peels of the fruits are rich sources of hydroxy cinnamic acid [Manthey, et. al., 2001].

Therefore, the present investigation was focused to evaluate the antimicrobial potential and phytochemical screening of seed extracts against common respiratory tract pathogens.

Material and methods

Collection and preparation of plant material

Seeds of *C. maxima* were collected from the fruits purchased from the market of Haridwar (Uttarakhand, India) during September, 2013. Collected seeds were washed using distilled water and dried at room temperature. Well dried seeds were crushed into the powder form using electric grinder.

Extract preparation

Plant extracts were prepared by immersing 100 g of powdered plant material in 300 mL (1:3) with four different organic solvents *i.e.* petroleum ether (PET), acetone (ACE), methanol (MeOH) and distilled water (H₂O). Loaded the powdery material in Soxhlet apparatus and extracted for 72 h by successive method [Ahmed et al., 1998]. Each preparation was filtered through a sterilized Whatman No. 1 filter paper. Each extract was evaporated with the help of vacuum evaporator at 30°C. The dried extracts obtained from vacuum evaporator were exposed to Ultraviolet rays for 24 h to checked sterility on nutrient agar plates. All

extracts were stored in a refrigerator at 4°C for further use [Aneja et al., 2010].

Test Microorganisms

Upper respiratory tract infections pathogens were selected for this exploration. Clinical bacterial strains of Gram positive (*Streptococcus pyogenes* MTCC 442, *Streptococcus pneumoniae* MTCC 655, *Staphylococcus aureus* MTCC 1144) and Gram negative *Pseudomonas aeruginosa* MTCC 2474, *Haemophilus influenzae* MTCC 3826. Two fungal pathogens *Candida albicans* (MTCC 227), *Aspergillus niger* (MTCC 921) were selected and all pathogens were procured from Institute of Microbial Technology (IMTECH), Chandigarh (India).

Antibacterial Activity

Antibacterial activity of seeds was determined by agar well- diffusion method [Ahmed *et al.*, 1998]. Stock cultures were maintained at 4°C on slopes of nutrient agar medium. Active cultures for experiments were prepared by transferring microbial inoculums from stock cultures to test tubes containing Mueller-Hinton Broth (MHB) for bacteria that were incubated at 37°C for 24 h. 100 µL of diluted inoculums of 10⁵ CFU mL⁻¹ [IP, 1996] of 24 h old cultures of test organisms were poured and mixed in Mueller Hinton Agar (MHA) medium. Medium was poured in to sterilized Petri plates and allowed to solidify for 5 – 10 min. A cork borer of 6 mm diameter was used to punch wells in medium and filled with 45 µL of 200 mg mL⁻¹ final concentration of extracts. DMSO (dimethyl sulphoxide) was used as negative control. Each extract was assayed in triplicate and the mean values were observed. The plates were incubated at 37°C for 24 h. The antibacterial activities were interpreted from the dimension of the diameter of inhibition zone calculated in millimetres as observed from clear zones adjacent the wells.

Minimum inhibitory concentrations (MICs)

MIC was done by using modified two fold serial dilution method [Aboaba *et al.*, 2006; Singh et al., 2016]. Sterilized nutrient broth was poured equally into each test tube. Bacterial cultures were prepared by Mcfarland's turbidity standard scale number 0.5 was poured in test tubes containing broth with normal saline and incubated for 6 h at 37°C for making a turbid suspension of the microorganism. After incubation, dilution of the culture in DMSO was ready until it matching with the turbidity (1.5 x 10⁶ cfu/mL) of the Mcfarland's scale. A solution of MeOH extract was serially diluted with broth to obtain the following concentrations 100, 50, 25, 12.5, 6.25, 3.12 and 1.56 mg/mL. From the above suspension equipped in DMSO, 0.1 mL was dispensed into the different concentration of the extract in nutrient broth. The test tubes were observed for turbidity after incubating at 37°C for 24 h. The lowest concentration of the extract

in the broth which showed no turbidity determined the MIC.

Antifungal activity

The antifungal potential of seeds extracts were determined by Poisoned food technique [Grover and Moore, 1962; Singh *et al.*, 2016; Nene and Thapliyal, 2002]. An aliquot of 250 mg/mL concentration of different seeds extracts were poured into Petri dishes and followed by adding 19 mL of melted Sabouraud dextrose agar (SDA) medium. 6 mm mycelial discs were punched using sterile cork borer from the margins of two to three days old culture of *A. niger* and put the disc in the core of agar plate. Percentage inhibition zone of mycelial growth was determined by measuring the relative growth of the test fungus in treatment and control by using the following formula.

$$I = [C - T] / C \times 100$$

Where I, C and T are denotes the percentage inhibition, mean growth rate of the control and the treatment, respectively. Rather than the extract aphoticin B was used as the reference drug for the comparison. Plates were incubated for 48 – 72 h at 25°C in BOD incubator. All tests were assayed in triplicate and mean value was observed.

Phytochemical Screening

All extracts were subjected to identify the chemical nature of phytochemical constituents present in extracts [Trease and Evans 1996, Scalbert 1991].

Test for Alkaloids

The test solution gave brown precipitate with the Dragendroff's reagent. The presence of brown precipitate showed positive test whereas absence of precipitate was negative.

Test for Flavonoids

2-5 drop of 1% NH₃ solution is added to the extract in a test tube. A yellow colour is observed that indicate the presence of flavonoids.

After addition of conc. HCl in MeOH extract of material, a red colour appeared which indicated the presence of flavonoids.

Test for Steroids

Extract was mixed with 3 mL chloroform and 2 mL concentrate H₂SO₄ was added from side of test tube. Colour of the ring at connection of two layers was noted if a red colour observed it confirmed the presence of steroids.

Test for Resins

An aliquot of 10 mL of diluted extract and 10 mL of 1% cupper acetate solution was added and the mixture was shaking vigorously. A separate green colour indicated the presence of resin.

Test for Saponins

Extracts was diluted with distilled water to 20 mL and this was shaken in a graduated cylinder for 15 min. If 1 cm layer of foam produced it indicates the presence of saponins.

Test for Tannins

1% FeCl₃ was added in the extract and colour was observed. A bluish black colour was appeared which disappeared on addition of dilute H₂SO₄ following a yellow -brown precipitate indicates the presence of tannins.

Test for Phenols

2 mL of extract added in alcohol with one drop of neutral Fe₂Cl₃ (5%) solution. Formation of blue colour indicates the presence of phenols.

Test for Lignins

Phloroglucinol with HCl were added in the test solution. Formation of pink colour indicates the presence of lignins.

Test for Terpenoids

In a test tube 5 mL of H₂O extract was mixed with 2 mL of CHCl₃ further 3 mL of concentrated H₂SO₄ is added to the test tube containing extract to form a layer. If terpenoids are present an interface with a reddish brown colouration is formed.

Test for Glycosides

1 mL of conc. H₂SO₄ is prepared in test tube 5 mL the extract and mixed with 2 mL of glacial CH₂CO₂H containing 1 drop of FeCl₃. The above mixture is carefully added to 1 mL of concentrated H₂SO₄ so that the concentrated H₂SO₄ is underneath the mixture.

Test for proteins and amino acids

50 mg of extract was dissolved in 5 mL of distilled water and filtered through the Whatman filter paper no. 1. The filtrates of each extract were subjected to the following tests.

Biuret test

2 mL of filtrate was treated with few drops of 2% CuSO₄ solution. To this, 1 mL of 95% C₂H₅OH was added. If a pink colour is formed in ethanol (C₂H₅OH) layer it indicated the presence of proteins.

Result and Discussion

Antibacterial activities of seeds extracts of *C. maxima* are presented in Table 1. MeOH extract showed highest antibacterial activity among all solvents followed by ACE, H₂O and PET. Maximum inhibition zone was found against *S. aureus* (24±0.88 mm) followed by *S. pneumoniae* (21.78±0.36 mm), *H. influenzae* (19.74±0.22 mm), *P. aeruginosa* (18.54±0.62), *S. pyogenes* (10.93±0.69 mm) and *C. albicans* (7.66±0.32 mm). Borah *et al.* studied antibacterial activity of ethanolic extracts of *C. maxima* against *S. aureus*, *E. coli* and *P. aeruginosa*. Antibacterial activities of the phytochemical constituents of the pericarp, mesocarp and segment membrane crude ethanolic extracts of *C. maxima* fruits were tested against *E. coli* and *S. typhimurium* [Borah *et al.*, 2012]. The antibacterial activity of the ethanol extract of *C. maxima* leaves against *E. coli* and *P.*

aeruginosa was investigated by Das *et al.* [Das *et al.*, 2013]. Abirami *et al.* reported the *in vitro* antibacterial activity of methanol extracts of leaves, peel and pulp of *C. hystrix* and *C. maxima* (red and white) fruit extract against *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, *S. typhi* and *E. coli*. MeOH extract of leaves and pulp were found to have maximum activity compared to peel extracts against all microorganisms [Abirami *et al.*, 2013]. The antibacterial activity of the volatile constituents of *C. maxima* (fruit epicarp) against *Bacillus pumilus*, *B. subtilis*, *Staphylococcus aureus*, *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Salmonella typhi* was evaluated by Pandey *et al.* [Pandey *et al.*, 2010].

The MICs values for MeOH extract were observed between 3.12 mg/mL to 25 mg/mL (Fig. 1). MIC value was observed against *S. aureus* at 3.12 mg/mL followed by *S. pneumoniae* and *P. aeruginosa* at 6.25 mg/mL. *H. Influenzae*, *S. pyogenes* at 12.5 mg/mL and 25mg/mL for *C. albicans*.

Antifungal activity of seeds extracts is presented in table 2. Percentage inhibition was observed maximum with 37.01% of H₂O extract followed by MeOH (22.47%), PET (8.36%) and ACE (1.56%). The control mycelia growth diameter was determined between 34.23±0.46 to 35.4±0.28 mm. amphotericin B

showed 67.08% inhibition used for comparison of seed extract.

Phytochemical screening of MeOH and ACE extracts showed the presence of flavonoids, amino acids/proteins, alkaloids, tannins, glycosides. The phytochemical screening of *C. maxima* reported the presence of alkaloids, glycosides, saponins, steroids, phenols, flavonoids, proteins and amino acids MeOH extract, glycosides, lignins, steroids, terpenoids, phenols, flavonoids, proteins and amino acids in ACE extract, glycosides, saponins, flavonoids, tannins, proteins and amino acids in H₂O while PET extract showed steroids terpenoids and alkaloids presented in table-3. The root bark contains β-sitosterol and several acridone alkaloids, and coumarins which show antimicrobial activity. Citrus flavonoids have a large spectrum of biological activity including antibacterial, antifungal, antidiabetic, anticancer and antiviral activities [Burt, 2004; Oruno, 2006]. Carbohydrates like phytol, synephrine, methyl antranilate, fuctose, glucose and pectin are reported in the leaf, flowers and peel [Jantan *et al.*, 1996; Shi 1992; Wang, 1979; Palasiri; 1948].

Extractive yield of *C. maxima*

The maximum yield was found with H₂O (9.3%) extraction followed by MeOH (11.5%), ACE (2.4%) and PET (1.8%) respectively are presented in Table 1.

Table 1. Extractive yield, colour and physical state of concentrated crude extract of *Citrus maxima* (seeds)

Extract	Weight of sample (gm)	Weight of extract (gm)	% yield (w/w)	Colour	Consistency
PET	200	3.6	1.8	Yellowish,	Waxy
ACE	200	4.8	2.4	Brownish	Waxy
MeOH	200	11.5	5.75	Reddish	Fludy sticky
H ₂ O	200	18.6	9.3	Brownish, blackish	Oily

Table -2 Diameter of inhibition zone all seeds extracts of *C. maxima*

Pathogen	Inhibition zone (in diameter)				Erythromycin
	PET	ACE	MeOH	H ₂ O	
<i>S. pneumoniae</i>	18.33±0.57	21.78±0.36	16.40±0.84	17.34±0.70	22.56±0.95
<i>S. aureus</i>	18.88±0.22	14.73±0.80	24±0.88	12.22±0.62	29.42±0.73
<i>H. influenzae</i>	10.11±0.21	19.74±0.22	13.24±0.80	10.39±0.21	21.68±0.43
<i>P. aeruginosa</i>	12.63±0.91	18.54±0.62	14.98±0.28	12.71±0.41	16.91±0.19
<i>S. pyogenes</i>	10.93±0.69	15.80±0.25	14.2±0.47	14.04±0.33	25.01±0.97
<i>C. albicans</i>	7.66±0.32	8.34±0.51	9.55±0.46	8.89±0.58	26.55±0.56

Where, given values cork borer diameter (6 mm), all values are mean of three replicates, PET = Petroleum ether, ACE= Acetone, MeOH = Methanol, H₂O= Aqueous

Table – 3 Effect of seeds extract and amphotericin B on the mycelial growth rate of *A. niger*

Fungicide/extract	Mycelial growth (mm)	Control	% inhibition
PET	32.44±0.58	35.4±0.28	8.36
ACE	33.3±0.28	33.86±0.34	1.65
MeOH	28.6±0.76	36.89±0.45	22.47
H ₂ O	21.56±0.54	34.23±0.46	37.01
amphotericin B	11.44±0.56	34.76±0.34	67.08

Cork borer diameters 6 mm, all values are mean ± standard error, all values are three replicates

Table-4. Phytochemicals screening of crude seeds extracts of *C. maxima*

S. No	Extracts	Tests			
		Petroleum ether (PET)	Acetone (ACE)	Methanol (MeOH)	Aqueous (H ₂ O)
1.	Alkaloids	+	-	+	-
2.	Glycosids	+	+	+	+
3.	Lignins	+	+	-	-
4.	Tannins	-	-	-	+
5.	Saponins	+	-	+	-
6.	Steroids	-	+	+	+
7.	Terpenoids	-	+	-	-
8.	Phenols	+	+	+	+
9.	Flavanoids	-	+	+	+
10.	Proteins and amino acids	-	+	+	+
11.	Resins	-	-	+	-

where + = present and - = absent

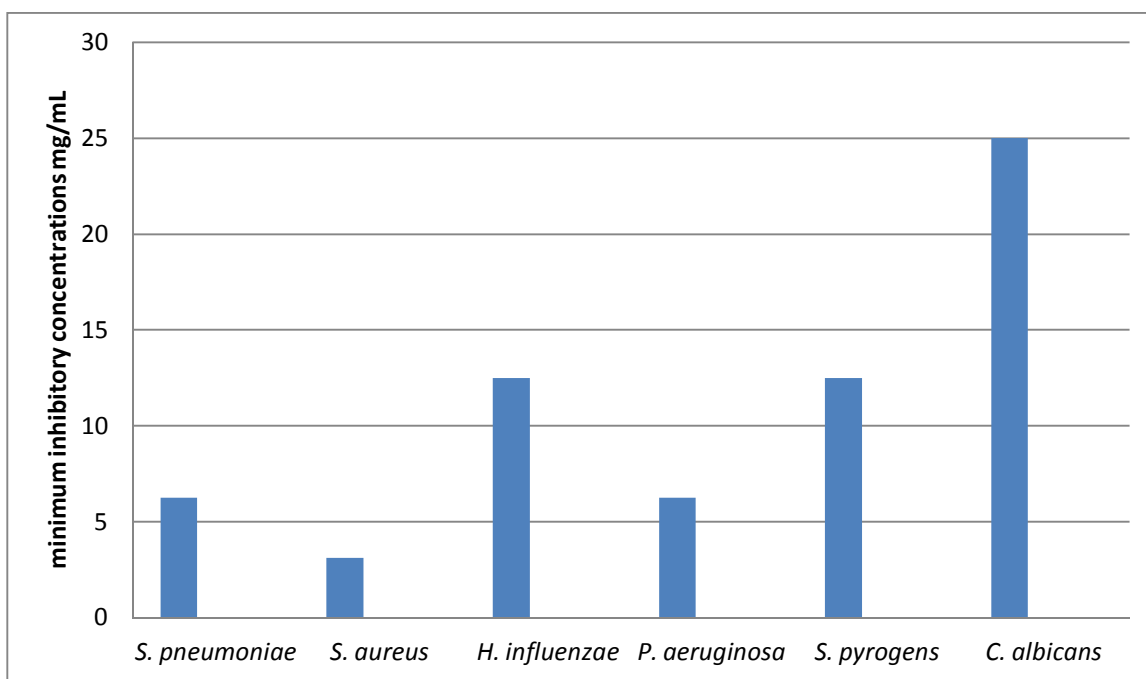


Fig 1. Minimum inhibitory concentration (MIC) of MeOH extract of *C. maxima*. The minimum inhibition is observed at (a) 12.5 mg/mL against *H. influenzae* and *S. pyogenes* (b) 3.12 mg/mL against *S. aureus* (c) 6.25 mg/mL against *S. pneumoniae* and *P. aeruginosa* and *C. albicans* 25 mg/mL.

Conclusion

Present study concluded that the seeds of *C. maxima* have potent antimicrobial activity against respiratory pathogens. The MeOH extract found most potent in comparison to other solvents. Preliminary phytochemical test revealed the presence of phenols, tannins, and saponins which may be responsible for the antimicrobial, found in crude seeds extracts.

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1. Abirami, A, Nagarani, G, and Siddhuraju, P. Antibacterial activity of crude extract of *Citrus hystrix* and *Citrus maxima*, *Inte J Pharma Sci and Res*, 2013;4(1): 296-300.
2. Aboaba, O.O., Smith, S.I., Olide, F.O. Antimicrobial effect of edible plant extract on *Escherichia coli* 0157:H7. *Pak. J. Nutr.* 2006; 5(4): 325-327.
3. Ahmed I, Mehmood Z, Mohammad F, Screening of some Indian medicinal plants for their antimicrobial properties, *J Ethnopharma.* 1998; 62: 183-193.
4. Aneja, K. R., Joshi, R., Sharma, C. Potency of *Barleria prionitis* L. Bark extracts against oral diseases causing strains of bacteria and fungi of clinical origin, *N Y Sci J.* 2010; 3(11):5-12.
5. Baomin, P.Y. Chemical constituents of peels of *Citrus grandis*, Shenyang Yaoke Daxue Xuebao. 2000; 17 (15): 332-333.
6. Baroh, M., Ahmed, S., Das, S. A. Comparative study of the antibacterial activity of the ethanolic extracts of *Vitex negunda* L., *Fragaria vesca* L., *Terminalia arjuna* and *Citrus maxima*, *Asi J Pharma and Bio Res.* 2012; 2(3): 183-187.
7. Barrion, A.A., Mabesa, R.C, Dizon, E.T. and Hurtada, W.A. Antibacterial activity of crude ethanolic extracts of pummelo [*Citrus maxima* (Burm.) Merr.] on *Listeria monocytogenes* and *Staphylococcus aureus*, *The Asi Int J of L Sci*, 2013; 22 (2):503-14.
8. Bhavnani, S.M., Ballow, C.H. New agents for Gram-positive bacteria, *Curr Opin Microbiol*, 2000; 3: 528-534.
9. Bisset, N.G. Herbal Drugs and Phytopharmaceuticals. 1994; CRC Press, Boca Raton.
10. Burt, S.A. Essential oils: their antibacterial properties and potential applications in foods: A review, *Inte J F Micro.* 2004; 94: 223-253.
11. Chan-Yenug, M, Lam, S. Occupational asthma. *An Rev Respir Dis.* 1986; 133: S686-703.
12. Chariandy, C.M, Seaforth, C.E, Phelps, R. H. Screening of medicinal plants from Trinidad and Tobago for antimicrobial and insecticidal properties. *J. Ethnopharmacol.* 1999; 64: 265-270.
13. Cowan, M.M. Plant products as antimicrobial agents, *Clin Microbiol Rev.* 1999; 12 (4):564-582.
14. Das, K., Tiwari, R.K.S., Shrivastava, D.K. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends, *J Med Plants Res.* 4, 2010; (2):104-111.
15. Das, S., Baroh, M., Ahmed, S. Antibacterial Activity of the Ethanolic extract of Leaves of *Citrus Maxima* (Burm.) Merr. On *Escherichia coli* and *Pseudomonas aeruginosa*. *Asi J Pharma and Clini Res.* 2013; 6(4): 136 – 139.
16. Dubey, D., Padhy, R.N. Antibacterial activity of *Lantana camara* L. against multidrug resistant pathogens from ICU patients of a teaching hospital. *J. Herb Med.* 2013; 3:65-75.
17. Evans, W.C. Trease and Evans Pharmacognosy, 5th edi. 1996. Harcourt Brace and Company, India.
18. Gautam, S.S., Navneet and Kumar, S. The antibacterial and phytochemical aspects of *Viola odorata* Linn. Extracts against respiratory tract pathogens. *Proc. Natl. Acad. Sci., India B Biol. Sci.* 2012; 82(4):567-572.
19. Grover, R.K., Moore, J.D. Toximetric studies of fungicides against brown rot organisms, *Sclerotia fructicola* and *S. laxa*. *Phytopathol.* 1962; 52:876-880.
20. I.P., Indian Pharmacopeia. Vol. 2, CSIR, Ministry of Health, India. 1996; A-100-107.
21. Jadhav, A., Sameer, M., Sathe, S., Sonawane, A. and Kadam, V. Microscopical, Physicochemical and Phytochemical Screening of *Citrus maxima* Peel. *Indo Amer J Pharma Res.* 2013; 3(8): 6430-6435.
22. Jantan, I., Said, A.A., Ahmad, A.R., Ali, N.A.M., Norsiha, A. Chemical composition of some *Citrus* oils from Malaysia. *J Esse Oil Res.* 1996; 8(6):627-632.
23. Kayser, F.H., Morenzoni, G., Santanam, P. The second European collaborative study on the frequency of antimicrobial resistance in *Haemophilus influenzae*. *Eur J Clin Microbiol Infect Dis.* 1990; 9: 810-817.

24. Khare, C.P. Indian medicinal plants, An Illustrated Dictionary, New Delhi; 2007. Springer (India) Private Limited.
25. Kundusen, S., Gupta, M., Mazumder, U.P., Haldar, P.K, Panda, S.P. and Bhattacharya, S. Exploration of anti-inflammatory potential of *Citrus limetta* Risso and *Citrus maxima* (J. Burm) Merr. *Pharmacologyonline*. 2011; 1:702-09.
26. Manthey, A. and Grohmann, K. Concentrations of hesperidin and other orange peel flavonoids on citrus processing byproducts. *J. Agri. Food Chem*. 1996; 44: 811–814.
27. Manthey, A. and Grohmann, K. Phenols in citrus peel by-products. Concentrations of hydroxycinnamates and polymethoxylated flavones in *Citrus* peel molasses. *J. Agri. Food Chem*. 2001; 49: 3268–3273.
28. Morton, J.F. Fruits of Warm Climates; Creative Resources Systems, Inc; Winterville, NC, USA, 1987; 147-151.
29. Nabakishore, N., Padhy, R.N. and Singh, P. K. Evaluation of antibacterial and antioxidant efficacy of the fern *Azolla carolinana* symbiotic with the Cyanobacterium *Anabaena azolleae*. *Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci*. 2015; 85(2): 555-569.
30. Nene, Y., Thapilyal, L. (Poisoned food technique of fungicides in plant disease control. (3rd Ed.) Oxford and IBH Publishing Company. 2002; New Delhi.
31. Ortuno, A.A., Baidez, P., Gomez, M.C., Arcas, I., Porras, A.G. and Del Rio J.A. *Citrus paradise* and *Citrus sinensis* flavonoids: Their influence in the defence mechanism against *Penicillium digitatum*. *F Chem*. 2006;98(2): 351-358.
32. Palasiri, U. Preliminary studies on pectin of *Citrus maxima*. *J Pharma Asso of Siam*. 1948; 2 (1): 18-24.
33. Pandey, R. R., Dubey, R.C., and Saini, S. Phytochemical and antimicrobial studies on essential oils of some aromatic plants. *Afri J. Biotech*. 2010; 9(28):4364-4368.
34. Prabhat, Navneet, and Krishna, S. Antibacterial activity of apamarga *Achyranthes aspera* Linn. *Nat. Acad. Sci. Lett*. 2005; 28:379 – 381.
35. Rath, S., Padhy, R.N. Surveillance of multidrug resistance of 10 Enteropathogens in a hospital and in vitro efficiency of 25 Ethnomedicinal plants used by an Indian aborigine. *Asi Pacif J Trop Dis*. 2012; 2(spl vol): S336-S346.
36. Kumar, S., Saini, S., and Dubey, R.C. Antimicrobial activity of von – volatile essential oils of certain medicinal plants against some entric bacterial pathogens. *J. Sci. Trans. Environ. Technov*. 2015; 9(1): 1-4.

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