

Medicinal and Phytochemical Analysis of Alcoholic Whole Fruit Extracts of *Actinidia Deliciosa*

Soham S. Mulye¹, Anand S. Maurya¹, Swapnil A. Kamble¹, Padma V. Deshmukh¹, Lal Sahab Yadav²,
Raghav K. Mishra³, Ashish Jain^{1*}

¹ Department of Microbiology, Smt. Chandibai Himathmal Mansukhani College, Ulhasnagar -421003, Thane, Maharashtra, India

² Department of Botany, Smt. Chandibai Himathmal Mansukhani College, Ulhasnagar -421003, Thane, Maharashtra, India

³ Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi -221005, U.P., India

*ajcdri@gmail.com, sohammulye@gmail.com

Abstract: Present study investigates antimicrobial, antioxidant activity and phytochemical profiling of alcoholic extracts of whole fruit of *Actinidia deliciosa* (kiwi fruit). Cold and hot extracts of whole *Actinidia deliciosa* fruit prepared in methanol and ethanol and their different concentrations were tested against common nosocomial infections and human pathogens of skin, gastro-intestinal tract and upper respiratory tract. Extracts were evaluated for their phytochemical constituents and antioxidant activity. Cold ethanolic extract of whole kiwi fruit has good antimicrobial activity as it inhibited the growth of almost all test organisms at concentration less than 20 mg/ml. Cold ethanolic extract was also found to be effective against *Candida albicans* at a concentration of 38.33 mg/ml. Antioxidant activity was examined by DPPH radical scavenging assay, highest antioxidant activity was displayed by cold methanolic extract. The qualitative phytochemical analysis of the extracts revealed the presence of alkaloids, flavonoids, saponins, cardiac glycosides, tannins and terpenoids in whole fruit of *Actinidia deliciosa*.

Index Terms: *Actinidia delicosa*, Antioxidant, Antimicrobial activity, Chromatography, Alcoholic extract, Phytochemicals, kiwi Fruit

I. INTRODUCTION

Fruits are the natural source of antimicrobial agents and antioxidants which can be added into our diet for prevention of diseases caused by pathogenic organisms (Joshi & Kumar, 2012). In order to carry out these reactions and proper functioning of body we need ample of nutrients which are supplied by the fruits. Kiwi fruit or Chinese gooseberries are the edible berries of several species of woody vines in the genus *Actinidia*. Kiwi fruit is oval in shape, greenish brown in colour, it has little sweet and bitter taste. It has many health

benefits like it helps in digestion, boosts our immunity, maintains blood pressure (Tyagi & Sahay, 2015). Kiwi fiber has a significant capacity to reduce the rate of diffusion of glucose. Kiwi fruit has potential of improving functions of gastro-intestinal (GI) tract and controlling GI tract infections. They are also rich source of Vitamin C and thus act as a powerful antioxidant eliminating the free radicals that could cause inflammation or cancer (Singletary, 2012). Antioxidants are the molecules that inhibit oxidation of cell contents like carbohydrates, proteins, DNA etc (El K ichaoi & El-Hindi, 2015). The concentration of natural biologically active compounds like alkaloids, tannins, flavonoids etc. are found to be very high in fruits (El Zawawy, 2015). The extensive use of antibiotics has resulted in emergence of antibiotic resistant organisms. There is need and demand for generating safer and natural therapeutic products for improving health and preventing diseases. Recent study has confirmed that addition of kiwi fruit in our diet can affect nutrition uptake. Actinidin present in it assist in breaking down proteins, improves digestion of protein and enhances absorption (Richardson, 2018). Not only the fruit but its leaves and roots have some medicinal properties. Significant work has been done on kiwi leaves and roots but no significant study previous to this research has been reported on whole kiwi fruit. The present study deals with antimicrobial, antioxidant activity and phytochemical screening of alcoholic extracts of whole kiwi fruit.

II. MATERIALS AND METHODS

A. Plant material

Fresh fruits of *Actinidia deliciosa* were bought from APMC market in Navi-Mumbai, Maharashtra State, India in the month

of April, 2017 and were identified in the Department of Botany, Smt. CHM College, Ulhasnagar-3. The whole fruits of *Actinidia deliciosa* were properly washed under tap water. Fruits (2 kg) were chopped into small pieces and were oven dried at 50 °C. Further the dried fruits were ground to fine powder. Powder was then stored in air tight containers for further studies.

B. Chemicals and reagents

1,1-diphenyl-2-picrylhydrazyl (DPPH) (99%) was purchased from Sigma-Aldrich. Ethanol, methanol and all other chemicals, reagents and solvents used in study were of analytical grade and purchased from Thomas Baker, unless stated otherwise. Nutrient agar and Sabouraud dextrose agar (SDA) were purchased from Hi-media (India).

C. Extraction of the fruit of *Actinidia deliciosa*

1) Cold extraction

For cold extraction, 30 g of dried fruit powder was submerged in 300 ml of respective solvents in shaking condition for 50 hours at room temperature in an orbital shaker (REMI). The extracts were separated from the residues by filtering through Whatman No. 1 filter paper. The extracts were concentrated and evaporated to dryness under reduced pressure at 50°C (El-Manhel & Niamah, 2015; Zhang, 2018; Ewansiha & Garba, 2012).

2) Hot extraction

For hot extraction, 30 g of dried fruit powder was extracted with each of the solvents absolute ethanol, and absolute methanol under reflux using Soxhlet apparatus (Borosil). The residual solvent was evaporated to dryness at 50°C (Zhang, 2018; Azwanida, 2015). The dried crude concentrated extracts were weighed to calculate the yield and stored in a refrigerator (4 °C), until used for analyses.

3) Yield of Extracts

Yield after extraction was calculated using following formula:

$$\% \text{ yield} = (\text{m extract} / \text{m dried sample}) \times 100$$
 where m extract, m dried sample are the mass of extract and mass of the dried sample, respectively.

D. Antimicrobial potential of extracts

1) Test cultures

The following MTCC strains were procured from IMTECH, Chandigarh, India. *Staphylococcus aureus* 3160, *Escherichia coli* 1885, *Pseudomonas aeruginosa* 1688, *Salmonella (enterica) typhi* 734 and *Candida albicans* 3017. *Streptococcus pyogenes* and *Corynebacterium diphtheriae* were clinical isolates procured from Microbiology department of Smt. CHM College, Ulhasnagar-3.

2) Culture media and Preparation of Inoculum

Stock cultures of each of the bacterial strains were prepared by inoculating pure cultures onto nutrient agar (Hi-Media, India) plates and incubated for 18 to 24 h at 37 °C to obtain colonies and pure culture of *Candida albicans* on Sabouraud's dextrose agar (Hi-Media, India) for 48 hours at 25 °C. The stock cultures

were maintained at 4°C on agar slants. Prior to sensitivity testing, culture inoculum was prepared by suspending the stock cultures in a sterile saline solution (0.85% NaCl) and adjusted to a turbidity of 0.5 MacFarland standards (1×10^6 CFU/ml).

3) Sample preparation

The methanolic and ethanolic extracts were dissolved in 25% solution of Dimethyl Sulfoxide (DMSO) prepared in sterile saline to facilitate the dissolution of the extracts in the agar.

4) Preliminary screening of antibacterial potential of extracts by Ditch Plate Method

Preliminary screening of antimicrobial extracts was performed using agar ditch method (Spooner & Sykes, 1972; Wheat, 2001). In this method a ditch of 1cm x 5 cm dimensions was aseptically made in the sterile nutrient agar plates using a sterile scalpel. In 5 ml of sterile molten nutrient agar butt 0.25 g of respective extract (50 mg/ml) was added, mixed and poured into the ditch, it was allowed to solidify. Loopful of inoculum of each organism was streaked on the agar plate at right angle to ditch. The nutrient agar plates containing bacterial cultures were incubated in incubator at 37 °C for 24 hours. The susceptibility of microorganisms was determined by observing the growth on the agar surface on and around the ditch.

5) Preliminary screening of antifungal potential of extracts by Agar well diffusion method

The antifungal potential of extracts was tested using agar well diffusion method (Sen & Batra, 2012; Salvador & Pereira, 2003; Magaldi, 2004). Preliminary screening was carried out at concentration of 50 mg/ml. Sterile Sabouraud dextrose agar (SDA) plates were prepared and allowed to solidify on a leveled surface. Plates were swabbed with *Candida albicans* 3017 and using a sterile cork borer (diameter 9 mm) wells were punched into the plates. 0.25g of extract was added in 5ml of 25% DMSO (50 mg/ml). 120µl of solution was added into the wells using micropipette. The plates were kept undisturbed for 30 minutes at room temperature for diffusion. Since *Candida albicans* is a human pathogen plates were incubated at 37° C for 48 hours. The susceptibility of test organism was determined by observing the zone of inhibition on the agar around the well.

6) Determination of Minimum inhibitory concentration (MIC) by agar well diffusion method

Microorganisms found susceptible in preliminary screening were tested at various concentrations ranging from 0.5 to 50 mg/ml with the interval of 5mg/ml for determination of Minimum inhibitory concentration (MIC) of different alcoholic extracts of whole fruit of *Actinidia deliciosa* by agar well diffusion method. In this method 20 ml molten nutrient agar butt was cooled down to 40 °C and then 1 ml bacterial suspension was poured into it and properly mixed. The molten agar with bacterial suspension was poured into a sterile petri plate and was allowed to set and harden. For fungal culture sterile Sabouraud dextrose agar plates were prepared and were allowed to solidify. Plates were swabbed uniformly with fungal isolate using sterile swab. Wells of 9 mm diameter were punched aseptically using a

sterile cork borer on each agar plates prepared previously. Agar plates of each microorganism were prepared. In each well 120µl of respective extract was loaded at different concentrations and kept at room temperature for 30 minutes to allow diffusion of extract in the agar. 25% DMSO was served as control. The nutrient agar and Sabouraud dextrose agar plates were then incubated at 37 °C for 48 hours. The lowest concentration (highest dilution) of the extract that produced a visible clear zone of inhibition in the first 24 h when compared with the control wells was considered as initial MIC. The plates were incubated further for 24 h at 37 °C. The lowest concentration that produced zone of inhibition after a total incubation period of 48 h was regarded as final MIC (Magaldi, 2004; Vasireddy 2018; Haltalin, 1973).

E. Antioxidant activity of extracts by DPPH scavenging assay

The antioxidant activity of whole kiwi fruits extract was examined using 1, 1'-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method (Prakash, 2009). DPPH solution (0.05mM) was prepared freshly in methanol in amber coloured bottle and kept in refrigerator at 4 °C till further use. In this assay, 3.6 mL previously prepared methanolic DPPH solutions was added to 0.4 mL of methanolic or ethanolic extracts at different concentrations ranging from 10 mg/ml to 50 mg/ml with interval of 10 mg/ml and the contents were stirred vigorously for 15 sec. Then the solutions were allowed to stand at dark place at room temperature for 30 min for reaction to occur. Absorbance was measured against a blank at 517 nm with a double beam (Analytical Technologies PVT Limited SPECTRO 2080) and standard Ascorbic Acid was used as positive control. The percentage of DPPH radical-scavenging activity was calculated as:

$$\text{Percentage inhibition of DPPH radical} = \frac{A_0 - A}{A_0} \times 100$$

Where, A_0 is the absorbance of the control solution (containing all reagents except fruit extracts); A is the absorbance of the DPPH solution containing kiwi fruit extract.

F. Primary phytochemical screening by TLC

Phytochemical screening of the extracts was carried out for detection of different phytoconstituents including alkaloids, tannins, cardiac glycosides, saponins, terpenoids and flavonoids. The presence of phytochemical compounds was detected using Thin Layer Chromatography. For detecting the presence of phytochemicals the initial concentration of extracts selected was 10mg/ml and 10µl sample was loaded on to the TLC sheets. Extracts were loaded on Silica gel 60 F254 TLC aluminium sheets (5×7.5 cm) (Merck) were used to perform analysis (Aliyu, 2011; Bhole, 2015; Szymulanska- Ramamurthy, 2017). Details of solvent systems, developing reagents and positive

observations are as mentioned in the table below and are different for according to phytochemical.

III. RESULTS AND DISCUSSIONS

A. Preparation of Extracts

A total of four extracts of whole Kiwi fruit viz; Hot ethanolic extract (HEE), Cold ethanolic extract (CEE), Hot methanolic extract (HME) and Cold methanolic extract (CME) were prepared using the dried powder of whole Kiwi fruit. A greenish brown jell-like concentrate was recovered after extraction. All the extracts were labeled and stored at 4 °C for further use. Preparation of extracts was done using two solvents; absolute ethanol and absolute methanol and two extraction techniques were carried out: hot extraction and cold extraction and yields of the same are shown in Table II. Significant difference in the yield of both the extracts is not observed because both the solvents are polar in nature. Although slightly higher yield of extract with ethanol was in accordance with the previously published research work (Bushera, 2009).

Phytochemical	Solvent System	Developing reagent	Observation
Alkaloids	Ethyl acetate: Chloroform: Methanol (20:20:10)	Dragendroff's Reagent	Orange spots
Tannins	n Butanol: Acetic acid: Water (4:1:5)	10% FeCl ₃ in methanol and water (1:1)	Grey spots
Cardiac Glycosides	Chloroform: Methanol (9:1)	Dintrobenzoic acid	Brick red spots after incubation at 60-70°C for 5 minutes
Saponins	Choloroform: Glacial acetic acid: Methanol: Water (64:32:12:8)	0.5ml Anisaldehyde + 10ml Glacial acetic acid + 85ml Methanol + 5ml H ₂ SO ₄	Red spots in visible light
Terpenoids	Ethyl acetate: Acetic acid: Formic acid: Water (100:11:11:26)	Anisaldehyde sulphuric acid	Bluish violet spots
Flavanoids	Ethyl acetate: Formic acid: Water (8:1:1)	15ml of 3% Boric acid + 5ml of 10% Oxalic acid	Flourescent green spots in UV light

Table I. Phytochemical Screening of extracts: Details of solvent systems, developing reagents and positive observations.

Extracts	Yield of Extracts in (g) (from 30 g of powder)	Percent yield of extracts (%) (from 30 g of powder)
HEE	3.789	12.63
CEE	3.698	12.32
HME	3.296	10.98
CME	3.105	10.35

Table II. Yield of different whole fruit extracts of *Actinidia deliciosa*. HEE : Hot Ethanolic Extract, CEE : Cold Ethanolic Extract, HME : Hot Methanolic Extract, CME : Cold Methanolic Extract

B. Preliminary screening of extracts against test cultures by Agar Ditch Method.

The microorganisms chosen for study are common pathogens of skin infections (*Staphylococcus aureus* and *Streptococcus pyogenes*) like impetigo, carbuncle etc. (Lindenmayer, 1998), nosocomial infections (*Candida albicans* and *Pseudomonas aeruginosa*) (Khan, 2009; Jahani & Saeide, 2005), gastro-intestinal tract infections (*Escherichia coli* and *Salmonella typhi*) (Pradeep, 2008) and upper respiratory tract infections (*Corynebacterium diphtheriae*) (Pappenheimer, 1983). The preliminary antimicrobial screening was carried out using initially 50 mg/ml concentration of each extract. In primary screening all four extracts were found effective at 50 mg/ml concentration in inhibiting the growth of all pathogenic organisms selected for the study. The extract was concluded to be positive if the growth was not observed on and or around the ditch. All the extracts inhibited gram positive as well as gram negative organisms thus it shows broad spectrum activity. The results of the same are presented in Table-III and Figure-1.

Microorganisms	HEE	CEE	HME	CME
<i>S. aureus</i>	+	+	+	+
<i>S. pyogenes</i>	+	+	+	+
<i>C. diphtheriae</i>	+	+	+	+
<i>E. coli</i>	+	+	+	+
<i>P. aeruginosa</i>	+	+	+	+
<i>S. typhi</i>	+	+	+	+
<i>C. albicans</i>	+	+	+	+

Table III. Preliminary screening of whole fruit extracts of *Actinidia deliciosa* at 50 mg/ml concentration by ditch plate technique. Key: (+) = Inhibition of Growth. HEE: Hot Ethanolic Extracts, CEE: Cold Ethanolic Extracts, HME: Hot Methanolic Extracts, CME: Cold Methanolic Extracts.



Figure 1: Representative image of Preliminary Screening of extracts for Antimicrobial activity by Ditch Plate Method.

C. Determination of Minimum inhibitory concentration

MICs of different extracts were determined using a concentration range of 0.5 mg/ml to 50 mg/ml. The results for the same are depicted in Table IV and Figure-2. CEE inhibited all bacterial pathogens at concentration less than 20 mg/ml except *Salmonella typhi* (30 mg/ml). CME also had MIC against all bacterial pathogens at a concentration less than 22 mg/ml except *Pseudomonas aeruginosa* (43.33 mg/ml) and *Salmonella typhi* (45 mg/ml). HEE and HME also showed good antibacterial activity at slightly higher concentration than cold alcoholic extracts. HEE showed MIC against all selected pathogens in a range from 25 to 38.33 mg/ml. HME exhibited MIC at a range of 18.33 to 48.33 mg/ml. Cold alcoholic extracts were more effective against selected bacterial pathogens whereas ethanolic extracts were more effective against fungal pathogen *Candida albicans*.

Culture	HEE MIC (mg/ml)	CEE MIC (mg/ml)	HME MIC (mg/ml)	CME MIC (mg/ml)
<i>S. aureus</i>	25± 2.88	15± 2.88	26.66± 1.66	18.3± 1.66
<i>S. pyogenes</i>	31.6± 1.66	18.33± 1.66	18.33± 4.40	11.6± 4.40
<i>C. diphtheriae</i>	26.6± 1.66	15± 2.88	35± 2.88	16.6± 3.33
<i>E. coli</i>	35± 2.88	16.6± 3.33	45± 2.88	21.6± 1.66
<i>P. aeruginosa</i>	31.6± 4.40	13.3± 3.33	48.3± 1.66	38.3± 1.66
<i>Salmonella spp</i>	35± 2.88	30± 2.88	46.6± 3.33	45± 2.88
<i>C. albicans</i>	38.3± 1.66	38.33± 1.66	45± 2.88	48.3± 1.66

Table IV. Minimum Inhibitory Concentration (MIC) of different whole fruit extracts of *Actinidia deliciosa* in mg/ml by agar well diffusion method. HEE: Hot Ethanolic Extracts, CEE: Cold Ethanolic Extracts, HME: Hot Methanolic Extracts, CME: Cold Methanolic Extracts. Values expressed as (Mean ± SE) are average of three values.

From above results it can be concluded that CEE was found most potent in inhibition of bacterial pathogens and fungal pathogen as well. The results of Minimum inhibitory concentration of extracts were expressed as mean of three replicates together with standard errors. Statistical calculations were done by Microsoft Excel 2010.

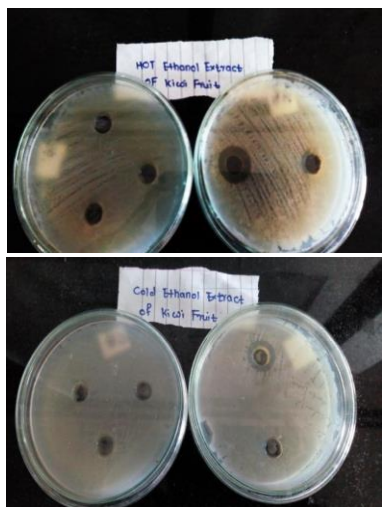


Figure 2: Representative image of Minimum Inhibitory Concentration of Hot and Cold Ethanolic Extracts by agar well diffusion method against *Candida albicans*.

D. Antioxidant activity of extracts

All the prepared extracts of kiwi fruit were subjected to antioxidant screening or free radical scavenging using the DPPH method. All the extracts showed antioxidant activity at 10 mg/ml concentration. The methanolic extracts showed better antioxidant activity. Results of antioxidant activity are summarized in Figure-3, where for both cold and hot alcoholic extracts, the higher the concentration of extracts the

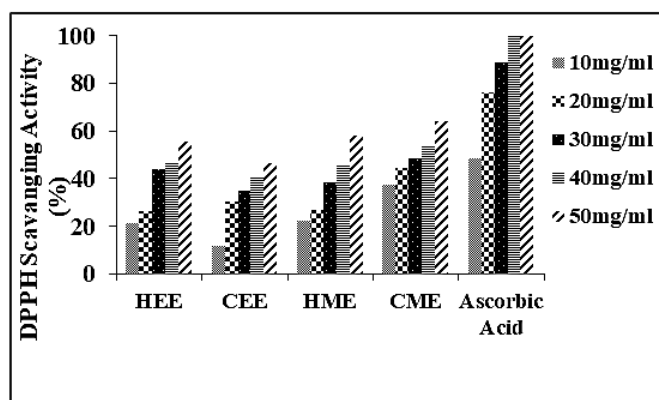


Figure 3. Percentage antioxidant activity of different whole fruit extracts of *Actinidia deliciosa* by DPPH scavenging assay. HEE : Hot Ethanolic Extracts, CEE : Cold Ethanolic Extracts, HME : Hot Methanolic Extracts, CME : Cold Methanolic Extracts

higher is the DPPH scavenging activity. In the present study, the order of scavenging activity of alcoholic whole fruit extract of *Actinidia deliciosa* extracts at 10% concentration is as follows: CME > HME > HME > CEE. Here at 50 mg/ml

concentration of different extracts maximum 64.25 % inhibition was observed with CME and minimum 46.47% inhibition was recorded with CEE.

E. Primary phytochemical screening by TLC

Thin layer chromatography (TLC) is generally performed for a better identification of the phytochemicals. Results of primary phytochemical screening revealed the presence of alkaloids in all the four extracts whereas, terpenoids were detected in three extracts except CEE. Flavonoids and tannins were present in cold extracts prepared out of ethanol and methanol whereas; saponins were found only in HEE. Cardiac glycosides were detected in HEE and CME. In the present study the TLC profiling of different alcoholic extracts shows the presence of different phytochemicals in different extracts. The results of the same are presented in table Table V and Figure-4. It was observed that among the four extracts CME was found effective in extracting maximum number of secondary metabolites. Alkaloids, tannins and flavonoids were common in cold extracts. After phytochemical profiling of different alcoholic extracts of whole fruit of *Actinidia deliciosa* revealed the presence of alkaloids, tannins, flavonoids, glycosides, saponinins and terpinoids. Hence it can be concluded that the kiwi fruit has a rich phytochemicals profile.

Phytochemicals	HEE	CEE	HME	CME
Alkaloids	+	+	+	+
Tannins	-	+	-	+
Flavonoids	-	+	-	+
Cardiac glycosides	+	-	-	+
Saponins	+	-	-	-
Terpenoids	+	-	+	+

Table V. Preliminary phytochemical screening of different whole fruit extracts of *Actinidia deliciosa*.

Key : “+” = Presence of phytochemical, “-”= Absence of phytochemical. HEE : Hot Ethanolic Extracts, CEE : Cold Ethanolic Extracts, HME : Hot Methanolic Extracts, CME : Cold Methanolic Extracts.

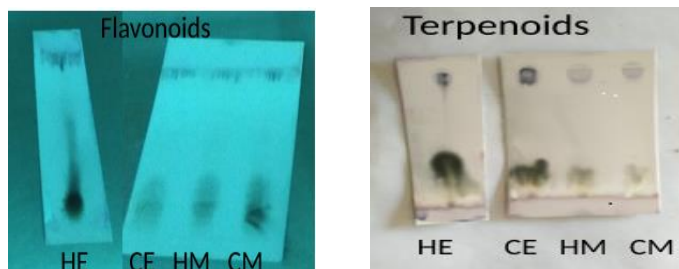


Figure 4. Representative images of Phytochemical screening of extracts by Thin Layer Chromatography using Silica gel 60 F254 TLC aluminium sheets.

CONCLUSION

Present study was aimed to evaluate the antioxidant potential and analysis of phytochemical constituents of whole fruit alcoholic extracts of *Actinidia deliciosa* and assessment of its effectiveness against various bacterial and fungal human pathogens. Different alcoholic extracts showed broad spectrum activity against a panel of microorganisms by inhibiting bacterial and fungal pathogens. Extracts were effective in inhibiting the growth of both Gram positive and Gram negative organisms responsible for various human infections. The fruit showed potential for development of drugs against many diseases. All the extracts possess significant antioxidant activity, the property which suggests potential use of these extracts as anti-inflammatory elements and for anticancer studies. Findings of phytochemical analysis ensure that whole fruit of *Actinidia deliciosa* can accumulate many secondary metabolites of medicinal value. Thus, the whole fruit can be exploited as a natural source for harvesting phytochemicals by using advance techniques of extraction, screening, identification and separation. These results explore possibilities of finding out antimicrobial compounds in *Actinidia deliciosa* fruit to prove its clinical potential in treating diseases. Further active constituents of the extracts can be purified for better results and can be evaluated for compared effectiveness with market available compounds and drugs. These extracts can be used for formulation of drugs against varied range of pathogens. The current work is the first ever report on whole fruit of *Actinidia deliciosa*, used for solvent extraction and exploring its medicinal potential.

ACKNOWLEDGEMENT

Authors are thankful to Department of Microbiology, Smt. CHM College, Ulhasnagar-3 for providing laboratory facilities to carry out this study. Authors would also like to thank Dr. Sunanda Karandikar for her keen interest and enthusiasm in the project.

REFERENCES

Aliyu, A. B., Musa, A. M., Abdullahi, M. S., Ibrahim, H., & Oyewale, A. O. (2011). Phytochemical screening and antibacterial activities of *Vernonia ambigua*, *Vernonia blumeoides* and *Vernonia ocephala* (Asteraceae). *Acta Poloniae Pharmaceutica-Drug Research*, 68 (1), 67-73.

Al-Manhel, A., & Niamah, K. A. (2015). Effect of aqueous and alcoholic plant extracts on inhibition on some type of microbes causing spoilage of food. *Journal of nutrition and food sciences*, 5(5), 1-3.

Azwanida, N. N. (2015). A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. *Med Aromat Plants*, 4, 196.

Bhole, R. P., Shinde, S. S., Chitlange, S. S., & Wankhede, S. B. (2015). A High-Performance Thin Layer Chromatography (HPTLC) Method for Simultaneous Determination of

Diphenhydramine Hydrochloride and Naproxen Sodium in Tablets. *Analytical Chemistry Insights*, 10, 47-51.

Bushera, S., Farooq, A., & Muhammad, A. (2009). Effect of extraction solvent/ technique on antioxidant activity of selected medicinal plants. *Molecules*, 14, 2167-2180.

El Kichaoi, A., & El-Hindi, M. (2015). The antimicrobial effects of fruit extracts of *Punica granatum*, *Actinidia deliciosa* and *Citrus maxima* on some Human Pathogenic Microorganisms. *American International Journal of Biology*, 3(2), 69-75.

El Zawawy, N. A. (2015). Antioxidant, Antitumour. Antimicrobial studies and quantitative phytochemical estimation of ethanolic extracts of selected fruit peels. *International Journal of Current Microbiology and Applied Sciences*, 4(5), 298-309.

Ewansiha, J. U., & Garba, S. A. (2012). Antimicrobial activity of *Cymbopogon Citratus* and its phytochemical properties. *Frontiers in Science*, 2(6), 214-220.

Haltalin, K. C., Markley, A. H., & Woodman, E. (1973). Agar plate dilution method for routine antibiotic susceptibility testing in a hospital laboratory. *Am J Clin Pathol*, 60(3), 384-394.

Jahani, S., & Saeide, S. (2005). Investigating the antibacterial effects of plant extracts on *Pseudomonas aeruginosa* and *Escherichia coli*. *International journal of infection*, 3(2), 1-4.

Joshi, V. K., & Kumar, A. (2012). Antimicrobial, Antioxidant and phytochemical screening from fruit and vegetable wastes. *International journal of food fermentation technology*, 2(2), 123-136.

Khan, R., Islam, B., & Akram, M. (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.

Lindenmayer, J. M., Schoenfield, S., & Grady, R. (1998). Methicillin-resistant *Staphylococcus aureus* in a high school wrestling team and the surrounding. *Arch Intern. Med*, 158(8), 895-899.

Magaldi, S., Mata-Essayag, S., Hartung de Capriles, C., Perez, C., Colella, M. T., Olaizola, C., & Ontiveros, Y. (2004). Well diffusion for antifungal susceptibility testing. *International Journal of Infectious Diseases*, 8, 39-45.

Pappenheimer, A. M. Jr., & Murphy, J. R. (1983). Studies on the molecular epidemiology of diphtheria. *Lancet*, 2(8356), 923-926.

Pradeep, B. V., Manojbabu, M. K., & Palaniswamy, M. (2008). Antibacterial activity of *Punicagranatum L.* against Gastro Intestinal Tract Infections causing organisms. *Ethnobotanical Leaflets*, 12, 1085-1089.

Prakash, V., Mishra, P. K., & Mishra, M. (2009). Screening of medicinal plant extracts for antioxidant activity. *Journal of medicinal plants*, 3(8), 608-612.

- Richardson, D. P., Ansell, J., & Drummond, L. N. (2018). The nutritional and health attributes of kiwifruit. *European Journal of Nutrition*, 57, 2659–2676.
- Salvador, M. J., & Pereira, P. (2003). Comparative study of antibacterial and antifungal activity of Callus culture and adult plants extracts from *Alternanthera maritima* (Amaranthaceae). *Brazilian Journal of Microbiology*, 34, 131-136.
- Sen, A., & Batra, A. (2012). Antimicrobial activity of different solvent extracts of medicinal plants. *International Journal of current pharmaceutical research*, 4(2), 67-73.
- Singletary, K. (2012). Kiwifruit: overview of the potential health benefits. *Nutr Today*, 47(3), 133–147.
- Spooner, F. D., & Sykes, G. (1972). Laboratory assessment of antibacterial activity. In: Norris E, Ribbons DN, editors. *Methods in Microbiology*. London: Academic Press.
- Szymulanska-Ramamurthy, K. M., Zhao, M., & Che, C. (2017). Phytochemical study of *Illicium angustisepalum* and its biological activities. *Acta pharmaceutica Sinica B*. 7(4), 485-590.
- Tyagi. S., Nanher, A.H., & Sahay, S. (2015). Kiwi fruit: Health benefits and medicinal importance. *Rashtriya krishi*, 10(2): 98-100.
- Vasireddy, L., Bingle, L.E.H., & Davies, M. S. (2018). Antimicrobial activity of essential oils against multidrug-resistant clinical isolates of the *Burkholderia cepacia* complex. *PLoS ONE*, 13(8), e0201835.
- Wheat, P. F. (2001). History and development of antimicrobial susceptibility testing methodology. *J Antimicrob Chemother*, 48(1), 1–4.
- Zhang, Q. W., Lin, L. G., & Ye, W. C. (2018). Techniques for extraction and isolation of natural products: a comprehensive review. *Chinese Medicine*, 13, 20-45.
