International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 7; Issue 5(H); May 2018; Page No. 12787-12790
DOI: http://dx.doi.org/10.24327/ijcar.2018.12790.2261

Research Article

COMBINATION EXTRACT OF CALENDULA OFFICINALIS AND PSIDIUM GUAJAVA ON ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY

Abhilasha Sawant*., Madhuri Shinde and Pallavi Dhekale

Department of Pharmaceutical Chemistry, Gourishankar Institute of Pharmaceutical and Education Research, Limb, Satara, 415015, Maharashtra, India

A R T I C L E I N F O

Article History:

Received 9th February, 2018
Received in revised form 26th March, 2018 Accepted 17th April, 2018
Published online 28th May, 2018

Key words:

Antibacterial and antifungal activity, agar well diffusion assay

A B S T R A C T

The present study was carried out to evaluate the antimicrobial and antifungal activity of combine ethanolic extracts of Calendula officinalis and Psidium guajava against two clinically important bacteria and fungi namely Staphylococcus aureus, E. coli andAspergillus flavus, Candida glaberata.The antimicrobial and antifungal activity of combined extracts was done with agar well diffusion assay in plates containing nutrient agar media. The antimicrobial activity of ethanolic extracts showed that all the combinations of extracts were effective against the test microorganisms. The patterns of inhibition varied with the different plant part extracted as the tested microorganisms. The methanolic extract of Psidium guajava and Calendula officinalis showed effective antibacterial and antifungal activity. The combined extracts of Calendula officinalis (petals) and Psidium guajava (leaves) antibacterial and antifungal activity showed reduced zone of inhibition by 10 mm in Aspergillus flavus, 2 mm in Candida glaberata, 4 mm in Staphylococcus aureus and increased by 2 mm in E. coli while comparing with streptomycin and fluconazole.

INTRODUCTION

Antibiotics provide the basis for the fungal and bacterial infections therapy. The discovery of antibiotics and making use of them as chemotherapeutic agents has made the medical fraternity to believe that they will eradicate various infectious diseases. However, indiscriminate use of antibiotics in human and veterinary healthcare systems has led to the emergence of multi-drug resistant (MDR) strains of different groups of microorganisms. The emergence and dissemination of MDR bacteria has made chemically synthesized antibiotics ineffective for the treatment of infectious diseases caused by such bacteria. These circumstances have propelled the researchers and scientists to explore new antimicrobial substances from various sources such as medicinal plants.[22,23,24] There are many studies that have described different type of plants such as herbs, shrubs and trees with the aim of knowing their phytoconstituents and using them for the treatment of various diseases as possible alternatives to the synthetic drugs. The screening of plants for medicinal purposes represents a serious effort to discover newer, safer, and possibly more effective drugs with the potential of fighting pathogenic bacteria and fungi. [6,7,8,9,10]

Corresponding author:* **Abhilasha Sawant
Department of Pharmaceutical Chemistry, Gourishankar Institute of Pharmaceutical and Education Research, Limb, Satara, 415015, Maharashtra, India

The green medicines are widely believed as safe and dependable in contrast with expensive synthetic drugs that have undesirable side effects along with beneficial effects. The plants have been in use in traditional medicine worldwide since long time but are still understudied, particularly in clinical microbiology. In past few decades, the curiosity to evaluate plants possessing antimicrobial, antifungal, anti-inflammatory activity for various diseases has grown many folds and a large number of biologically active compounds have been characterized. Several studies have established that many plants contain substances like peptides, tannins, alkaloids, essential oils, phenols, and flavonoids among others, which have antimicrobial properties.

Psidium guajava is member of the family Myrataceae, which is very common in the tropical countries and known by the English name Guava.[2] The Guava is a phytotherapic plant, which contains active components that help to treat various diseases like malaria, gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums, and a number of other conditions. The components present in Guava include lectins, phenols, tannins, flavonoids, essential oils, fatty acids, vitamins, etc. However, most of the medicinal properties of the Guava are attributed the presence of flavonoids. [2] The plant Calendula officinalis belongs to the family: Asteracea. Commonly known as English marigold or pot marigold, field marigold , garden marigold, gold bloom, holligold, maravilla and, marybud. The orange petals and the whole flower head are used medicinally.

Marigold flowers often used in food industry for their nutritive qualities as well as coloring of several culinary products, is well known of centuries in popular medicine because of their biologic activities.The major active constituents include saponins, triterpendiol esters and flavonoids including hyperoside and rutin, the orange flower contain a high content of caroteniodes including flavoxanthin and auroxanthin. Calendula has antibacterial and antifungal activity [1], and it has been used for the treatment of burns, abrasions, skin inflammations, ulcers, wounds and eczema. It has been used internally for the treatment of gastiritis, bleeding of duodenal ulcers and colitis.[21] Calendula officinalis for its inhibitory effects on the human immunodeficiency virus type1(HIV-1), calendula officinalis extracts show anti-cancer effects in vitro studies on tumor cell lines, derive from Leukemias, melanomas, fibrosacomas, breast, prostate, cervix, lung and pancreas The aim of this study was to evaluate the antimicrobial activity of ethanol extracts of Calendula officinalis and *Psidium guajava* to establish if they are effective in inhibiting the growth of bacteria and fungi such as Staphylococcus aureus, Escherichia coli, Aspergillus flavus and Candida glaberata. [12,15,17,18,19,20]

MATERIALS AND METHODS

Sample collection and extraction

Fresh leaves of *P. guajava* and flowers of Calendula officinalis were collected from the nurseries. Leaves and petals were washed gently under tap water and left to dry at room temperature for 2 days, the leaves of guava and petals of marigold were crushed separately to make powder.[3] 50 gram of the powder of *P. guajava* and *Calendula officinalis* were mixed separately with 200 ml ethanol in conical flasks. The flasks containing extracts were heated on water bath for 1 h and placed at room temperature for 5 days. The flasks were manually shaken daily to obtain maximum extraction. After 5 days, each extract added to centrifuge tubes and centrifuged at 4000 rpm for 10 min to separate the supernatant. The supernatant containing extracts of *P. guajava* and *Calendula officinalis* were transferred into pre-weighed beakers and were left to dry completely on water bath at 60°C to obtain an ethanol free extract residue of *P. guajava* and Calendula officinalis. [1,2,11,13,14,16]

Preparation of stock solutions

The dry powdered extracts of P. guajava and Calendula officinalis were dissolved separately in methanol to get the final concentration of 0.5 mg/ml for each extract.

Test organisms

Microbial strains were obtained from the microbiology laboratory of GIPER, Limb, Satara. In total four microorganisms were tested against the above mentioned plant extracts in which was Gram positive Staphylococcus aureus, Gram negative namely Escherichia coliand Aspergillus flavus, Candida glaberatafugal strains. The strains were maintained on nutrient agar slants at 4°C.[5]

Preparation of culture media

Nutrient Agar media was prepared by suspending 38 g in 1000 ml of distilled water. The media was sterilized by autoclaving at 121°C for 15 min and poured into sterile Petri plates at around 50°C. To observe the effect of pH on the growth of tested bacteria, pH was adjusted by adding 0.1M HCL or 0.1M NaOH into the media.

Antibacterial assay[1, 2, 11, 13, 14, 16]

The antimicrobial activity of ethanol extracts of P. guajava and Calendula officinaliswas done using the agar well diffusion assay as described earlier. Petri plates of 90 mm diameter were poured with agar media and allowed to solidify to make a base layer. The agar plates were marked to divide into required parts and labelled for specific organism, extract name, and pH. A fresh bacterial culture of 100 µl containing approximately 1 × 10^6 CFU/ml. CFU/ml of test microorganism was inoculated onto nutrient agar media plates and spread homogeneously using a glass spreader; the plates were incubated for 15 min at 37°C to complete dryness of media surface. Wells of 6 mm in diameter were punched off with the help of sterile borer in agar plates. Wells were then filled with the plant extract solution (ranging 10 µl–25 µl) in the combinations. Petri plates were placed for 30 min in refrigerator for diffusion of extracts and then incubated at temperatures 37°C for 24 hours. At the end of the incubation period, the zone of inhibition (including well diameter) was measured. The experiment was carried out thrice independently in duplicate and the mean of all the readings is mentioned in the results. The combined extract of *P. guajava* and *Calendula officinalis* were tested antibacterial activity at concentration 25 mg/µl each. Streptomycin 5 µg/disc was used as positive control and DMSO (solvent) used as negative control.All tests were done in triplicate.

Antifungal assay[1,2,11,13,14,16]

Antifungal activity was measured using methods of disc diffusion plates on agar. In order to test the antifungal activity, the fractions of two extracts were dissolved in 70% ethanol. 20 mL of Agar was poured into each 90 mm Petri dish. C. officinalis and P. guajava were grown in agar at 27◦C for 48 h. One hundred µL of suspension containing approximately 108 bacteria/mL was placed over agar in Petri dishes and dispersed. Then, sterile paper discs (6 mm diameter) were placed on agar to load 10 and 15 µL of each extract sample (1 mg/mL) and combined extract of *P. guajava* and *Calendula officinalis* were tested antibacterial activity at concentration 25 mg/µl each. One hundred units of fluconazole obtained from a local pharmacy, were used as a positive control and ethanol as a negative control. Inhibition zones were determined after incubation at 27◦C for 48 h. All tests were done in triplicate.

RESULT AND DISCUSSION [1, 2, 11, 13, 14, 16]

The ethanolic extracts of P. guajava and Calendula officinalis were tested for their antimicrobial activity in combinations of concentration. The tested plants have been in use as folk medicine and were familiar to the local people. The results of the antimicrobial activity of ethanolic extracts showed that all the concentrations were effective against tested microorganisms with varying zones of inhibition. The ethanolic extract of P. guajava was found to be inhibition against all bacteria and fungi. The diameter of zones of inhibition exhibited by S. aureus were 20 mm,E. coli 19mm and Aspergillus flavus 25mm and Candida glaberata 20 mm. The zone of inhibition for C. officinalis (petals) was observed inhibition against Aspergillus flavus 25 mm, Candida glaberata 20 mm, S. aureas15 mm, E. coil 18 mm.The combined extract of P. guajava and Calendula officinalis when tested at concentration 25 mg/µl each, was found most inhibitory to E.

coli showing zone of inhibition of 20 mm (Table 1).[4] This combination further inhibited growth of Candida glaberata fairly well (18 mm zone diameter); however, it was less effective in inhibiting the growth of Aspergillus flavus and S. aureus where zone diameter was observed only 15 mm and 16 mm respectively.

Table 1 Zone of inhibition: Antifungal and Antibacterial activity

Sr.no	Extracts	Aspergillus flavus	Candidaglaberata	S.aureas	E.coil
1	P. guajava (Leaves)	25 mm	20 mm	20 mm	19 mm
2	C. officinalis (petals)	25 mm	20 mm	15 mm	18 mm
3	Combine extracts(leaves and petals)	15 mm	18 mm	16 mm	20 mm

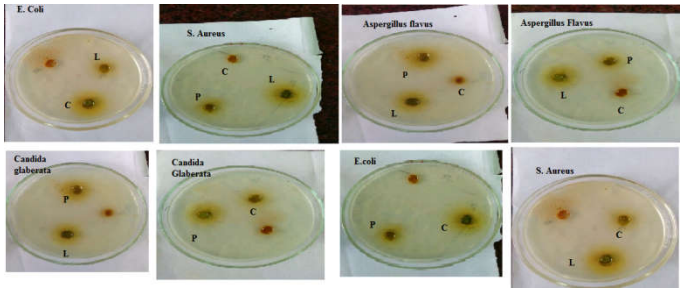

Figure 1 Typical agar plates showing the growth of inhibition zones by C. officinalis and P. guajava

Above figure showes P: C. officinalis petal extract, L: P. guajava leaves extract, C: combined extract of C.officinalis and P.guajava

CONCLUSION

This study highlights the synergistic therapeutic potentials of Calendula officinalis and *Psidium guajava* leaves ethanolic extracts in vitro against the clinically important strains of Gram-positive, Gram-negative bacteria and fungi. The ethanolic leaves extracts of *Calendula officinalis* and *P. guajava* exhibited clear zones of inhibition against the test bacteria and fungi indicating that the folk herbal drugs have great potentials as antimicrobial agents.[26,27,28,29] The synergistic effect from the association of different plant extracts against pathogenic bacteria will lead to new choices for the treatment of infectious diseases. It would be advantageous to standardize methods of extraction and *in vitro* testing so that the search could be more systematic and interpretation of results would be facilitated.

References

1. Efstratios Efstratiou, Abdulla Hussain, Poonam Nigam, Antimicrobial activity of C. Officinalis petal extracts against fungi, as well as Gram-negative and Gram-positive clinical pathogens: complementary therapies in clinical practice 18; 2012; 173-176.
2. PriyaGurnani, Ajith Krishnan, Antibacterial activity of guava leaves extract against Lactobacillus acidophilus: An In-Vitro Study: *International Journal of Oral Health and Medical Research*; March-April 2016; Vol 2; Issue 6.
3. Gitika and Manoj Kumar, Antibacterial activity of Psidium guajava leaves extracts against some gram-positive and gram-negative bacteria: *ejpmr*; 2016; 3(10); 261-266.
4. MeigyNelceMailoa, Meta Mahendradatta, Antimicrobial Activities Of Tannins Extract From Guava Leaves (Psidium Guajava L) On Pathogens Microbial; *International journal of scientific & technology research*; vol 3; issue 1; jan. 2014
5. JasminAraFarhana, Md. FarukHossain, AleyaMowlah, Antibacterial Effects of Guava (Psidium guajava L.) Extracts Against Food Borne Pathogens; *International Journal of Nutrition and Food Sciences* ; 2017; 6(1): 1-5
6. Abubakar E-MM. Antibacterial potential of crude leaf extracts of Eucalyptus camaldulensis against some pathogenic bacteria. *Afr J Plant Sci* 2010; 4:202-209.
7. Alviano DS, Alviano CS. Plant extracts: search for new alternatives to treat microbial diseases. *Curr Pharm Biotechnol* 2009; 10:106-121.
8. Karou D, Dicko MH, Simpore J, Traore AS. Antioxidant and antibacterial activities of polyphenols from ethnomedicinal plants of Burkina Faso. *Afr J Biotechnol* 2005; 4:823-828.
9. Kupeli E, Tosun A, Yesilada E. Assessment of anti-inflammatory and antinociceptive activities of Daphne pontica L. (Thymelaeaceae). *J Ethnopharmacol* 2007; 113:332-337.
10. Arora DS, Kaur J. Antimicrobial activity of spices. *Int J Antimicrob Agents* 1999; 12:257-262.
11. Okigbo RN, Omodamiro OD. Antimicrobial Effect of Leaf Extracts of Pigeon Pea (Cajanuscajan (L.) Millsp.) On Some Human Pathogens. *J Herbs, Spices Med Plants* 2006; 12:117-127.
12. Ganguli S. Neem: A therapeutic for all seasons. *CurrSci* 2002; 82:1304.
13. Subapriya R, Nagini S. Medicinal properties of neem leaves: a review. *Curr Med Chem* 2005; 5:149-146.
14. MahfuzulHoque MD, Bari ML, Inatsu Y, Juneja VK, Kawamoto S. Antibacterial activity of guava (Psidiumguajava L.) and Neem (Azadirachtaindica A. Juss.) extracts against foodborne pathogens and spoilage bacteria. *Foodborne Pathog Dis* 2007; 4:481-488.
15. El-MahmoodAM, Ogbonna OB, Raji M. The antibacterial activity of Azadirachtaindica (neem) seeds extracts against bacterial pathogens associated with eye and ear infections. *J Med Plants Res* 2010; 4:1414-1421.
16. Jaiarj P, Khoohaswan P, Wongkrajang Y, Peungvicha P, Suriyawong P, Saraya ML. Anticough and antimicrobial activities of Psidiumguajava Linn. Leaf extract. *J Ethnopharmacol* 1999; 67:203-212.
17. Abdelrahim SI, Almagboul AZ, Omer MEA, Elegami A. Antimicrobial activity of Psidiumguajava L. Fitoterapia 2002; 73:713-715.
18. Smania JA, Monache FD, SmaniaEdFA, Cuneo RS. Antibacterial Activity of Steroidal Compounds Isolated from Ganodermaapplanatum (Pers.) Pat. (Aphyllophoromycetideae) Fruit Body. *Int J Med Mushrooms* 1999; 1:325-330.
19. Sukanya SL, Sudisha J, Hariprasad P, Niranjana SR, Prakash HS, Fathima SK. Antimicrobial activity of leaf extracts of Indian medicinal plants against clinical and phytopathogenic bacteria. . *Afr J Biotechnol* 2009; 8:6677-6682.
20. Cowan MM. Plant Products as Antimicrobial Agents. *ClinMicrobiol Rev* 1999; 12:564-582.

21. Uniyal SK, Singh KN, Jamwal P, Lal B. Traditional use of medicinal plants among the tribal communities of ChhotaBhangal, Western Himalaya. *J. Ethnobiol. Ethnomed.* 2006; 2.
22. Del Serrone P, Nicoletti M. Antimicrobial activity of a neem cake extract in a broth model meat system. *Int J Environ Res Public Health* 2013; 10:3282-3295.
23. Chaturvedi MP, Bag A, Rawat V, Jyala NS, Satyavali V, Jha PK. Antibacterial Effects of Azadirachtaindica Leaf and Bark Extracts in Clinical Isolates of Diabetic Patients. *Nat J Integ Res Med* 2011; 2:5-9.
24. Sharma D, Lavania AA, Sharma A. In vitro comparative screening of antibacterial and antifungal activities of some common plants and weeds extracts. *Asian J ExpSci* 2009; 23:169-72.
25. Okemo PO, Mwatha WE, Chhabra SC, Fabry W. The kill kinetics of Azadirachtaindica A. Juss (Meliaceae) extracts on Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Candida albicans. *Afr J SciTechnol* 2001; 2:113-118.
26. Mahmoud DA, Hassanein NM, Youssef KA, AbouZeid MA. Antifungal activity of different neem leaf extracts and the nimonol against some important human pathogens. *Braz J Microbiol* 2011; 42:1007-1016.
27. Gonçalves FA, Andrade Neto M, Bezerra JNS, Macrae A, Sousa OVd, Fonteles-Filho AA, et al. Antibacterial activity of GUAVA, Psidiumguajava Linnaeus, leaf extracts on diarrhea-causing enteric bacteria isolated from Seabob shrimp, Xiphopenaeuskroyeri (Heller). *Rev Inst Med Trop Sao Paulo* 2008; 50:11-15.
28. Vieira RHSdF, Rodrigues DdP, Gonçalves FA, MenezesFGRd, Aragão JS, Sousa OV. Microbicidal effect of medicinal plant extracts (Psidiumguajava Linn. and Carica papaya Linn.) upon bacteria isolated from fish muscle and known to induce diarrhea in children. *Rev Inst Med Trop Sao Paulo* 2001; 43:145-148.
29. Gnan SO, Demello MT. Inhibition of Staphylococcus aureus by aqueous Goiaba extracts. *J Ethnopharmacol* 1999; 68:103-108.

How to cite this article:

Abhilasha Sawant *et al* (2018) 'Combination Extract of Calendula Officinalis and Psidium Guajava on Antibacterial and Antifungal Activity', *International Journal of Current Advanced Research,* 07(5), pp. 12787-12790.
DOI: http://dx.doi.org/10.24327/ijcar.2018.12790.2261

****