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Antibacterial Potential of *Ocimum sanctum* and *Bryophyllum pinnatum* Against Wound Pathogens

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Abstract
Microorganisms are believed to play a significant role in impaired healing of chronic wounds which affect millions of people around the world. This problem is increased by emergence of multidrug resistant micro-organisms, especially *Staphylococcus aureus* and *Pseudomonas aeruginosa* present in hospital and community acquired infections. Medicinal plants are rich in phytochemical contents and having antimicrobial properties. **Aim:** Thus, aim of the present work was to investigate the antibacterial activity of *Ocimum sanctum* and *Bryophyllum pinnatum* extract against wound pathogens *Staphylococcus aureus* and *Pseudomonas aeruginosa.* **Methods:** Antibiotic resistance profile of the wound pathogens as well as antimicrobial activity and phytochemical analysis of both the extracts was carried out. **Results:** Antibiotic resistance profile of wound pathogens showed that 80% of *S. aureus* were resistant to oxacillin while all *P. aeruginosa* (100%) were found to be resistant to Trimethoprim. Antimicrobial activity of both the extracts showed that out of five *S. aureus* 80% were found to be sensitive to *Ocimum sanctum* as well as *Bryophyllum pinnatum* extract. On the other hand only 40% *P. aeruginosa* were sensitive to both the extracts. **Conclusion:** Both the extracts were found to be synergistically active in combination with antibiotics against the wound pathogens. Phytochemical analysis showed that in *Ocimum sanctum* extract Alkaloids, Glycosides and Flavonoids were present. In *Bryophyllum pinnatum* extract Alkaloids, Flavonoids and Saponins were present.

Keywords
Bryophyllum pinnatum, *Ocimum sanctum*, Wound pathogens.

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INTRODUCTION:
Many medicinal plants have antimicrobial properties used in traditional Indian system of medicine. Natural products either as pure compounds or as standardized plant extracts provide unlimited opportunities for new drug [1]. Plants are rich in phytochemical contents present in all organs of the plant including roots, stems, buds, leaves, flowers and fruits. Besides this, microorganisms are believed to play a significant role in impaired healing of chronic wounds and the development of infection-related complications [2] which affect millions of people around the world. This problem is increased by emergence of multidrug resistant micro-organisms, especially *Staphylococcus aureus* and *Pseudomonas aeruginosa* present in hospital and community acquired infections [3] [4].

Use of traditional medicine is based on its accessibility, affordability and its firm embedment within the faith systems of people [5] [6]. It has been

estimated by WHO that at least 80% of the world population, mainly in the developing countries, still dependent on herbal medicines for their primary health care needs. It has been estimated that 70% of wound healing ayurvedic drugs are plant based, 20% of mineral based and remaining 10% consists of animal products as their base material [7].

Like other plant parts, leaves are also used for therapeutic purpose. It is being used as a source of medicinal agents for antibacterial, anti-helminthic, astringent, sedative and stimulant. A number of polyherbal preparations containing antimicrobial action have been scientifically proved to possess wound healing activity. Tulsi is an aromatic shrub in the basil family Lamiaceae that is thought to have originated in North central India and now grows native throughout the Eastern world tropics. In ayurveda, tulsi is known as "The Incomparable One," "Mother Medicine of Nature" and "The Queen of Herbs," and is revered as an "elixir of life" that is without equal for both its medicinal and spiritual properties. Within India, tulsi has been adopted into spiritual rituals and lifestyle practices that provide a vast array of health benefits that are just beginning to be confirmed by modern science. In addition to these health-promoting properties, Tulsi is recommended as a treatment for a range of conditions including anxiety, cough, asthma, diarrhea, fever, dysentery, arthritis, eye diseases, indigestion, hiccups, vomiting, gastric, cardiac and genitourinary disorders, back pain, skin diseases, ringworm, insect, snake and scorpion bites and malaria. It possesses anti-inflammatory, analgesic, immunostimulatory, free radical scavenging and antimicrobial activity. The free radical scavenging activity of plant flavonoids help in the healing of wounds. The topical wound healing activity of aqueous extract of leaves of *O. sanctum* has been reported [8].

Genus *Bryophyllum* of the family Crassulaceae is a valuable medicinal as well as ornamental plant. *Bryophyllum pinnatum* is the air plant, miracle leaf or life plant and is used in ethno medicine for treatment of earache, burns, abscesses, ulcer, insect bites and diarrhea. Alcoholic extracts of *B. pinnatum* showed antimicrobial activity against a number of Gram (+ve) and (-ve) bacterial strains [9]. Leaves of this plant possess disinfectant and anti-bacterial properties used for boils, swelling, insect-bite, burns and wounds [10]. Thus, aim of the present work was to investigate the antibacterial activity of *Ocimum sanctum* and *Bryophyllum pinnatum* extract against wound pathogens *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

MATERIALS AND METHODS:

I. **Collection of plant materials:** Leaves of *Ocimum sanctum* and *Bryophyllum pinnatum* were collected from botanical garden in Nagpur.

II. **Preparation of extract:** For the preparation of methanol extract *Ocimum sanctum* and *Bryophyllum pinnatum* leaves were rinsed with water and dried. The leaves were ground into fine particles and 10gm powder of each plant was added in 200ml distilled water in respective conical flasks. The conical flasks were kept in rotary shaker for 72 hours at room temperature. After 72 hours, it was filtered using Whatman's filter paper No.1 and then crude extracts obtained by filtration were used for further process [11] [12].

III. **Collection and identification of wound pathogens:** A total of 10 wound bacterial pathogens were collected from pathology laboratory and cultures were identified on the basis of morphological, cultural and biochemical characteristics [13].

IV. **Agar Well Diffusion Method:** Wound pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* strains were grown over night on nutrient agar at 37^0C, and the colonies were suspended in sterile saline water equivalent to a 0.5 McFarland standard (1.5×108CFU/ml). The suspension (100 µL) was spread over Mueller Hinton agar. The antibacterial activity of aqueous extracts of *Ocimum sanctum* and *Bryophyllum pinnatum* leaves was performed using well diffusion technique. The wells of 6mm diameter were cut into agar medium with a sterilized cork borer. Thereafter, 20µl each of the extracts were added separately into the separate wells. The plates were incubated at 37^0C for 24 hours. The diameter of the zone of inhibition around each well was measured and recorded [14].

V. **Disc Diffusion Method:** The antibiotic sensitivity test was carried out by Kirby-Bauer disc diffusion method. *S. aureus* and *P. aeruginosa* were tested for their sensitivity against 5 antibiotic discs each (Table 1 and 2). *P. aeruginosa* and *S. aureus* strains were grown over night on nutrient agar at 37^0C, and the colonies were suspended in sterile saline water equivalent to a 0.5 McFarland standard (1.5×108CFU/ml). The suspension (100 µL) was spread over the Mueller Hinton agar. The plates were kept at room temperature for 10 minutes for drying under strict aseptic condition. The antibiotic discs were carefully placed on to the

surface of the Muller Hinton agar plates using sterile forceps. The plates were incubated at 37^{0}C for 24 hrs. The agar plates were examined for zone of inhibition around the disc and the wound pathogens were classified as "resistant" or "sensitive" based on the standard interpretative chart according to Clinical and Laboratory Standards Institute (CLSI) guidelines [14] [15].

VI. **Synergistic activity of *O. sanctum* and *B. pinnatum* extracts with antibiotics against wound pathogens:** Synergistic activity of *Ocimum sanctum* and *Bryophyllum pinnatum* leaves aqueous extract was evaluated in combination with antibiotic discs. Lawn of the wound pathogens were prepared as described before. On Mueller Hinton Agar, wells of 6mm diameter were prepared in which antibiotic discs were placed aseptically. On each antibiotic disc of the well, 20µl of the extract was added. The plates were incubated at 37^0 C for 24 hours for zone of inhibition [16].

VII. **Phytochemical analysis of *O. sanctum* and *B. pinnatum:*** Phytochemical screening of the extracts was carried out according to methods described by [17] [18].

- **Test for Alkaloids:** To 2 ml of extract, 2 ml of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. Presence of green color or white precipitate indicates the presence of alkaloids.
- **Test for Flavonoids:** To 2 ml of extract, 1 ml of 2N sodium hydroxide was added. Presence of yellow color indicates the presence of flavonoids.
- **Test for Saponins:** To 2 ml of extract, 2 ml of distilled water were added and shaken in a graduated cylinder for 15 minutes. It resulted in the formation of 1 cm layer of foam that indicated the presence of saponins.
- **Test for Tannins:** To 1 ml of extract, 2 ml of 5% ferric chloride was added. Formation of dark blue or greenish black colour indicates the presence of tannins.
- **Test for glycosides:** To 2 ml of extract, 3ml of chloroform and 10% ammonia solution was added. Formation of pink color indicates presence of glycosides.

RESULTS AND DISCUSSION:

The present project was conducted to study the formulation and evaluation of antibacterial ointment against 10 wound pathogens such as *Staphylococcus aureus* (n=5) and *Pseudomonas aeruginosa* (n=5). The *Bryophyllum pinnatum* and *Ocimum sanctum* leaves extract were evaluated against these wound pathogens. The wound pathogens were tested against 10 different antibiotics (Table 1). Antibiotic resistance profile of wound pathogens showed that 80% of *S. aureus* were resistant to oxacillin while 100% were sensitive to Gentamycin (Table 3). Antibiotic resistant profile was carried out, 100% *P. aeruginosa* were found to be resistant to Trimethoprim while 100% were sensitive to Gatifloxacin and Ciprofloxacion (Table 4). In another study it was showed that *S. aureus* was resistant to Penicillin, Novobiocin, Methicillin, Vancomycin, Oxacillin and Tetracyclin [19].

It was found that out of five *S. aureus* 80% were found to be sensitive to *Ocimum sanctum* as well as *Bryophyllum pinnatum* extract. On other hand only 40% *P. aeruginosa* were sensitive to both the extract (Table 5 and 6). Another study showed that, the antibacterial effect of hot aqueous extract of *O. sanctum* varied from organism to organism [20]. The higher concentration was able to inhibit the growth of all pathogens under study. In another report it was showed that methanol, ethanol and hot water extract from the leaves of *Bryophyllum pinnatum* were tested against different wound isolates. It showed that methanol extract of both plants had a higher antimicrobial activity compared to their ethanol extracts, while the hot water extracts of both plants had no antimicrobial activity [21].

Synergistic activity of *Ocimum sanctum* extract with antibiotic discs against wound pathogens was carried out, in which extract was very much effective on *S. aureus* in combination with Gentamycin and Tetracycline (100% susceptibility each) while on *P. aeruginosa,* the extract was very much effective in combination with Ciprofloxacin and Gatifloxacin (100% susceptibility each) (Table 7 and 8). It was found that *Bryophyllum pinnatum* extract was synergistically very effective against *S. aureus* in combination with Tetracycline, Gentamycin and Erythromycin (100% susceptibility each) while on *P. aeruginosa* the extract was very much effective (100% susceptibility each) in combination with Ciprofloxacin and Gatifloxacin (Table 9 and 10).

Phytochemical analysis showed that in *Ocimum sanctum* extract Alkaloids, Glycosides and Flavonoids were present. In *Bryophyllum pinnatum* extract Alkaloids, Flavonoids and Saponins were present (Table 11). A phytochemical analysis is very useful in

the evaluation of some active biological compound of some medicinal plants. Previous study reported that Alkaloids, Saponins, Flavonoids and Tannins were revealed to be present in *Bryophyllum pinnatum*. This shows its possible medicinal values. The high Saponins content of *Bryophyllum pinmatum* justifies the use of the extract from this plant to stop bleeding and in treating of wounds. Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of Saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness [22]. The phytochemical screening in the previous study revealed that the extracts of *Ocimum sanctum* contain alkaloids, flavonoids, terpenes, saponins, glycosides. Studies suggest that these compounds are responsible for the potent antimicrobial effects of this plant. Tannins are responsible for the haemostatic and antidiaorrheal properties [23]. Flavonoids are responsible for antioxidant properties. Cardiac glycosides can act as cardiostimulants in cases of cardiac failure [24].

Table 1: Antibiotics used against *S.aureus*

Antibiotics	Concentration
Erythromycin	15 mcg/disc
Gentamycin	10 mcg/disc
Oxacillin	1 mcg/disc
Penicillin	10units/disc
Tetracycline	30 mcg/disc

Table 2: Antibiotics used in the study against *P. aeruginosa*

Antibiotics	Concentration
Chloramphenicol	30 mcg/disc
Ciprofloxacin	10 mcg/disc
Clindamycin	2 mcg/disc
Gatifloxacin	5 mcg/disc
Trimethoprim	5 mcg/disc

Table 3: Antibiotic resistance profile of *S. aureus*

Wound pathogens	Tetracycline	Gentamycin	Erythromycin	Oxacillin	Penicillin
S. aureus 1	24 mm	13 mm	16 mm	R	15 mm
S. aureus 2	19 mm	16 mm	30 mm	R	31 mm
S. aureus 3	20 mm	23 mm	24 mm	R	36 mm
S. aureus 4	R	19 mm	R	R	R
S. aureus 5	20 mm	17 mm	27 mm	11 mm	21 mm

Where R = Resistant

Table 4: Antibiotic resistance profile of *P. aeruginosa*

Wound pathogens	Ciprofloxacin	Clindamycin	Chloramphenicol	Trimethoprim	Gatifloxacin
P. aeruginosa 1	16 mm	R	R	R	19 mm
*P. aeruginosa*2	32 mm	R	R	R	27 mm
P. aeruginosa 3	15 mm	18 mm	26 mm	R	24 mm
P. aeruginosa 4	15 mm	R	R	R	17 mm
P. aeruginosa 5	14 mm	17 mm	24 mm	R	20 mm

Table 5: Antibacterial activity of *Ocimum sanctum* and *Bryophyllum pinnatum* leaves extract against *S. aureus*

Wound pathogens	*Ocimum sanctum* extract	*Bryophyllum pinnatum* extract	Mixture
S. aureus 1	10 mm	13 mm	13 mm
S. aureus 2	11 mm	10 mm	14 mm
S. aureus 3	12 mm	11 mm	14 mm
S. aureus 4	R	R	R
S. aureus 5	12 mm	12 mm	12 mm

Table 6: Antibacterial activity of *Ocimum sanctum* and *Bryophyllum pinnatum* leaves extract against *P. aeruginosa*

Wound pathogens	*Ocimum sanctum* extract	*Bryophyllum pinnatum* extract	Mixture
P. aeruginosa 1	R	R	R
P. aeruginosa 2	R	R	R
P. aeruginosa 3	10 mm	16 mm	16 mm
P. aeruginosa 4	R	R	R
P. aeruginosa 5	10 mm	11 mm	11 mm

Table 7: Synergistic activity of *Ocimum sanctum* leaves extract with antibiotic discs against *S. aureus*

Wound pathogens	*Ocimum sanctum* leaves extract + Antibiotic				
	Tetracycline	Gentamycin	Erythromycin	Oxacillin	Penicillin
S. aureus 1	30 mm	18 mm	17 mm	R	15mm
S.aureus 2	23 mm	30 mm	30mm	R	32mm
S. aureus 3	27 mm	26 mm	25mm	R	36mm
S. aureus 4	24 mm	21 mm	R	R	R
S. aureus 5	29 mm	20 mm	27mm	12mm	22mm

Table 8: Synergistic activity of *Ocimum sanctum* leaves extract with antibiotic discs against *P. aeruginosa*

Wound pathogens	*Ocimum sanctum* leaves extract + Antibiotic				
	Ciprofloxacin	Clindamycin	Chloremphenicol	Trimethoprim	Gatifloxacin
P. aeruginosa 1	28 mm	R	R	R	29 mm
*P. aeruginosa*2	26 mm	R	11 mm	16 mm	28 mm
P. aeruginosa 3	18 mm	20 mm	26 mm	22 mm	27 mm
P. aeruginosa 4	16 mm	R	R	18 mm	25 mm
P. aeruginosa 5	17 mm	23 mm	29 mm	R	26 mm

Table 9: Synergistic activity of *Bryophyllum pinnatum* leaves extract with antibiotic discs against *S. aureus*

Wound pathogens	*Bryophyllum pinnatum* leaves extract + Antibiotic				
	Tetracycline	Gentamycin	Erythromycin	Oxacillin	Penicillin
S. aureus 1	28mm	12mm	16mm	R	R
S. aureus 2	30mm	30mm	34mm	R	31mm
S. aureus 3	26mm	22mm	31mm	R	37mm
S. aureus 4	23mm	22mm	28mm	R	R
S. aureus 5	29mm	22mm	27mm	12mm	22mm

Table 10: Synergistic activity of *Bryophyllum pinnatum* leaves extract with antibiotic discs against *P. aeruginosa*.

Wound pathogens	*Bryophyllum pinnatum* leaves extract + Antibiotic				
	Ciprofloxacin	Clindamycin	Chloramphenicol	Trimethoprim	Gatifloxacin
P. aeruginosa 1	29mm	R	10mm	11mm	27mm
P. aeruginosa 2	27mm	R	17mm	14mm	29mm

P. aeruginosa 3	17mm	29mm	27mm	15mm	25mm
P. aeruginosa 4	15mm	R	R	R	26mm
P. aeruginosa 5	22mm	24mm	29mm	R	28mm

Table 11: Phytochemical analysis of *Ocimum sanctum* and *Bryophyllum pinnatum* aqueous extract

Tests	*Ocimum sanctum*	*Bryophyllum pinnatum*
Alkaloids	+	+
Tannins	-	-
Glycosides	+	-
Flavonoids	+	+
Saponins	-	+

Where, + = Present; - = Absent

CONCLUSION:

The present study clearly indicates that *Bryophyllum pinnatum* and *Ocimum sanctum* are rich sources of phytochemicals having immense antioxidants potential. *Bryophyllum pinnatum* showed the presence of Alkaloids, Flavonoids and Saponins and *Ocimum sanctum* showed the presence of Alkaloids, Glycosides and Flavonoids. It is suggested that *Bryophyllum pinnatum* and *Ocimum sanctum* may be recommended as useful source to prepare natural bioactive products from which we can develop new antimicrobial drug.

REFERENCES:

1. Tambekar D. and Khante B.S., Evaluation of antibacterial properties of ethnomedicinal herbs used by Korkus in Melghat of India against enteric pathogens. International Journal of Pharma and Biosciences, 1: 1-7, (2010).
2. Misic A.M., Gardner S.E., Grice E.A., The wound microbiome: modern approaches to examining the role of microorganisms in impaired chronic wound healing. Advances in Wound Care, 3: 502-510, (2014).
3. Lakshmanan K.K., Sankaranarayanan A.S., Antifertility herbs used by the tribals in Anaikatty, Coimbatore District, Tamilnadu. Journal of Economic and Taxonomic Botany, 14: 171-173, (1990).
4. Jayaraman A., Abbasi T., Abbasi S.A., Green synthesis of gold nanoparticles with aqueous extracts of neem (*Azadirachta indica*). Research Journal of Biotechnology, 5(1): 75-79, (2010).
5. Galabuzi C., Agea J.D., Fungo B.L. and Kamoga R.M.N., Traditional medicine as an alternative form of health care system: a preliminary case study of Nangabo sub-county, Central Uganda. African Journal of Traditional Complementary and Alternative Medicine, 7: 11-16, (2010).
6. Kamboj V.P., Herbal medicine. Current Sciences, 78: 35-51, (2000).
7. Mittal A., Sardana S., Pandey A., Herbal boon for wounds. International Journal of Pharmacy and Pharmaceutical Sciences, 5:1-12, (2013).
8. Singh V., Amdekar S., Verma O., *Ocimum Sanctum* (tulsi): Bio-pharmacological Activities. Webmed Central Pharmacol., 1(10): 1-7, (2010).
9. Akinsulire O.R., Aibinu I.E., Adenipekun T., Adelowotan T., Odugbemi T., In vitro anti-microbial activity of crude extract from plants *Bryophyllum pinnatum* and *Kalanchoe crenata.* Afr J Tradit Complement Altern Med., 4(3):338-344, (2007).
10. Khare C.P., Indian medicinal plants an illustrated dictionary. Springer Publishers, (2007).
11. Cheeseborough M., Medicinal laboratory manual for tropical countries. Vol. II. London, United Kingdom: Elsevier Health Sciences, (2000).
12. Tambekar D.H., Khante B.S., Chandak B.R., Titare A.S., Boralkar S.S., Aghadte S.N., Screening of Antibacterial Potentials of Some Medicinal Plants from Melghat Forest in India. Afr. J. Trad. CAM., 6(3): 228-232, (2009).
13. Collee J.G., Marr W. Tests for identification of bacteria and laboratory control of antimicrobial therapy, Chapter 7 and chapter 8. In: Mackie & McCartney Practical Medical Microbiology, by Fraser A.G., Marmion B.P. and Simmons (Eds.) 14th ed. Collee J.G., 131-151. New York: Churchill Livingstone: New York, (1996).
14. Bauer A.W., Kirby W.M.., Sherris, J.C., Turck, M., Antibiotic Susceptibility testing by a standardized disk method. Am. J. Clin. Pathol., 4 5(4): 493-496, (1966).
15. CLSI, Performance standards for antimicrobial susceptibility testing: 17th Informational supplement, Approved standard M100-S17, Wayne, USA. Clinical and Laboratory Standards Institute, (2007).
16. Ahmad I., Aqil F., In vitro efficacy of bioactive extracts of 15 Medicinal plants against ESßL-producing multidrug-resistant enteric bacteria. Microbiol. Res., 162: 264-275, (2007).
17. Trease G.E., Evans M.D., A text book of Pharmacognosy, 13th Edn. Baillier, Tindal and Caussel, London. pp. 144 -148, (1989).
18. Roghini R., Vijayalakshmi K. Phytochemical screening, quantitative analysis of flavonoids and minerals in ethanolic extract of Citrus paradisi. International Journal of Pharmaceutical Sciences and Research, 4859-4864, (2019).

19. Handali S., Hosseini H., Ameri A., Moghimipour E. Formulattion and evaluation of an antibacterial cream from *O. coriniculata* aqueous extract. Jundishapur J Microbiol., 4(4): 255-260, (2011).
20. Kumar A., Rahal A., Chakraborty S., *Ocimum sanctum* (Tulsi): a miracle herb and boon to medical science- A review. International Journal of Agronomy and Plant Production, 4 (7): 1580-1589, (2013).
21. Obioma A., Chikanka A.T., Dumo I., Antimicrobial Activity of Leave Extracts of *Bryophyllum pinnatum* and *Aspilia africana* on Pathogenic Wound Isolates Recovered from Patients Admitted in University of Port Harcourt Teaching Hospital, Nigeria. Ann Clin Lab Res 5:(3): 185, (2017).
22. Okwu, D.E. Evaluation of the Chemical Composition of Indigeneous Spices and Flavouring Agents. Journal of Science, 7: 455-45 9, (2004).
23. Asquith T.N., Butler L.G. Interaction of condensed tannins with selected phyto chemistry. Scientific Research,25(7): 1591-1593, (1986).
24. Sood S., Narang D., Dinda A.K., Maulik S.K. Chronic oral administration of *Ocimum sanctum* linn. augments endogenous antioxidants and prevents isoproternol-induced myocardial necrosis in rats. J.Pharm Pharmacol., 57(1): 127 - 133, (2005).