

Study on Antibacterial Property of Selected Medicinal Plant for Wound Dressing Material

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Abstract: The main aim of the study is to select best herbal leaf for medical bandages which have antimicrobial properties and have wound healing property. For this purpose, extracts of five ecofriendly herbs Aakada (*Calotropis gigantean*), Guava (*Psidium guajava* linn), Marigold (*Calendula officinalis*), Parijat or Night jasmine (*Nyctanthes arbor-tristis*), Durva (*Cynodon dactylon*) were used. After drying the sample leaves, extraction was done in aqueous, ethanol and methanol medium as per standard recommended procedures. Antibacterial property was checked with agar well diffusion method against gram positive and gram negative bacteria i.e *S. aureus* and *E. coli*. Among the five selected plant leaves guava leaves shows best zone of inhibition (ZOI) for ethanolic and methanolic extract i.e 16.5mm and 17.5mm for Gram positive Bacteria- *S. aureus*. while aakda leaves shows 12.5mm and 14.5mm for Gram positive Bacteria- *S. aureus* and 11.0mm and 15.0mm for Gram negative Bacteria- *E. coli*. As *S. aureus* bacteria is presents on any type of wound so these extract will be suitable for wound healing purpose. As per previous research Aakda leaves also possess UV protecting property which recommends these guava and aakda leaves extract for wound healing purpose as the best medicinal plant.

Keywords: Eco-Friendly Herbs, Antimicrobial, Wound Healing, Bandage

1. Introduction

Microorganisms can be found almost everywhere in the environment from at a height of 32 km and in depth of 11km in the sea [1]. Bacteria can be categorized as pathogenic, odor causing and non pathogenic. Bacteria hinder the natural healing process of any type of wound. To overcome the aforesaid set back and to improve the healing process, medications along with the support of wound dressing can be applied. Most common wound dressing material is cotton. Cotton has the largest global market in the category of natural fiber [2, 3]. Also cotton is widely used fiber for apparel due to its comfort properties. However, there are two major drawbacks for cotton fiber. They are low crease resistance and degradation due to microbial intervention [4].

Textiles are considered as shelter of Microorganisms. Textiles are omnipresent and play an essential part in human society. Cloths may contain certain types of microbes, which has been recently discussed as clothing microbiology and the

effect and interaction of cloths with human skin microflora. Microorganisms cause odor and decrease in strength of material. Hence, antimicrobial finish is the most desired finish; to improve the shelf life of the cotton based biodegradable wound dressings. It will improve the wound healing environment. For antimicrobial finish, in general borax, boric acid, formaldehyde, aldehydes are used [5].

Plants are the main source of food and medicine. They always had played a great role since ancient times. Currently, 65- 80% of the total population of developing countries uses medicinal plants as remedies as per the WHO [6]. India is a land of agriculture and fertility, where numbers of plants are used for medicinal purposes. So, "Vaidyas" (traditional ayurveda based medicine and health care practitioners in India) use plant leaves and their parts for healing any illness since time immemorable. Each part of India is blessed with some special plants. Few of such zones are Eastern Himalayas, Western Ghats, Andaman and Nicobar Islands and many more. On record total 3000 plants are recorded but traditionally more than 6000 plants are in use in India [7].

Plants have the capacity to produce a large number of organic chemical compounds such as Terpenes, Phenolic compounds and Nitrogen-containing compounds [8]. These compounds are used for various medicinal purposes such as blood coagulation, anti-inflammatory, antibacterial purposes.

2. Background

Plants have served as valuable sources of life supporting and assisting ingredients for traditional medicines for millennia. It is estimated that there are 250,000 to 500,000 species of plants on Earth. A relatively small percentage (1 to 10%) of this stock is used directly or indirectly as food by both humans and other animal species [9]. Other than that, the next predominant use of plant kingdom is in medicinal use by human. Mother Nature has bestowed human society with a vast arena of enzymes and biomolecules in the form of self-defense mechanism in all kinds of plant and animal kingdom. Father of Indian ayurveda Charak had identified; every plant as of herbal importance and microbial potential [10]. Human beings are never able to utilize 100% of this immense probable species of plant. The percentage has even reduced in modern era of modern science. Plants produce a diverse array of secondary metabolites, many of which have antimicrobial activities against some pathogenic microorganisms that are implicated in enteric infections. Some of these compounds are constitutive, existing in healthy plants in their biologically active forms and they elicit chemotherapeutic or chemoprophylactic properties against a wide range of infectious enteric diseases [11].

Because of the pathogenic microorganisms are developing the resistance to common antibiotics, there is a need for the search of new antimicrobial agents mainly among plant extracts [12, 13]. Potential effort has been the screening of more effective, affordable and readily available antimicrobial substances with diverse chemical structures and novel mechanisms of action from local medicinal plants or herbs [14, 15].

So, there is a huge gap between traditional knowledge and modern science. To reduce this difference a little bit, five well known plants has been selected for this study. The selected five plants are Aakada (*Calotropis gigantean*) shown in figure 1, Guava (*Psidium guajava linn*) shown in figure 2, Marigold (*Calendula officinalis*) shown in figure 3, Parijat or Night jasmine (*Nyctanthes arbor-tristis*) shown in figure 4, Durva (*Cynodon dactylon*) shown in figure 5.



Figure 1. *Calotropis gigantea* (Aakada). [25]



Figure 2. *Psidium guajava linn* (Guava). [26]



Figure 3. *Calendula officinalis* (Marigold). [27]



Figure 4. *Nyctanthes arbor-tristis* (Parijat). [28]



Figure 5. *Cynodon dactylon* (Durva). [29]

The plant leaves are locally available and are used for making natural antibacterial bandage. In the current research a comparative analysis had been done for selecting best antibacterial agents for making antibacterial bandage.

3. Material and Methodology

3.1. Chemicals Requirement

Distill Water, Ethanol, Microbes: gram positive *S. aureus* and gram negative *E. coli* (NTLC certified), Nutrient broth and all phytochemical testing reagents.

3.2. Preparation of Leaf Powder

Fresh leaves of Parijat or Night jasmine (*Nyctanthes arbor-tristis*) and Guava (*Psidium guajava linn*) were

collected from Burhanpur (MP). Similarly Akada (*Calotropis gigantean*), Durva (*Cynodon dactylon*) and Marigold (*Calendula officinalis*) were collected from Sendhwa and Indore respectively.

After collecting leaves, washed with clean water and then allowed to dry in shade for three weeks there after the dried leaves are grounded into fine powder in mixer grinder [16].

3.3. Preparation of Ethanol, Methanol and Water Extract

5.0g of leaves powder were macerated in 100ml of solvents (distilled water, laboratory grade Methanol and laboratory grade Ethanol, i.e. 5.0% (W/V)) for 24 hrs at room temperature [17]. (The maceration process took 24 hour at room temperature.). After this process filtration was done with Whatman filter paper No. 1. All the extracts were air dried in petri plates or oven dried at the temperature of 45°C (a) and the extracts were weighed. The filtrated were collected and % yield of above antibacterial agents were calculated.

3.4. Preparation of Nutrient Agar

Day1: Nutrient Agar was weighted 6.5g and Agar-Agar 7.5g, then dissolved in 500 ml D. water in conical flask. Put the flask in autoclave [18]. This solution was poured the petri

dish after 24hrs (day 2).

For Culturing Bacteria (Day 1): Nutrient broth 3.25g was dissolved in 100ml of D. water and 5-6 test tube were prepared by this solution. Put all the test tube in autoclave. Next day gram positive microbes *S. aureus* and gram negative microbes *E. coli* were mixed in 3-3 test tubes for growing culture in test tube. (Day 2).

Day 3: Petri dish and cultured bacteria were ready for used. Absorbents were also checked in UV-1900 for *S. aureus* and *E. coli*. which were 0.437 and 0.571 respectively.

3.5. Estimation of Antimicrobial Activity of Leaf Extract

The antimicrobial activity of the Water, Methanol and Ethanol extract of selected plant leaves were estimated against two microbial strains: *E. coli*, *S. aureus*.

3.6. Estimation of Zone Inhibition Using Well Diffusion Method

For each plant leaf two petri dishes were prepared each for gram positive and gram-negative strain. In every well 0.01mg/μl of antibacterial agents were poured and the solvent was used as control. The testing temperature was 37 C. After 24 hour the diameter of inhibition zones were measured [19]. Complete flow chart is shown in figure 6.

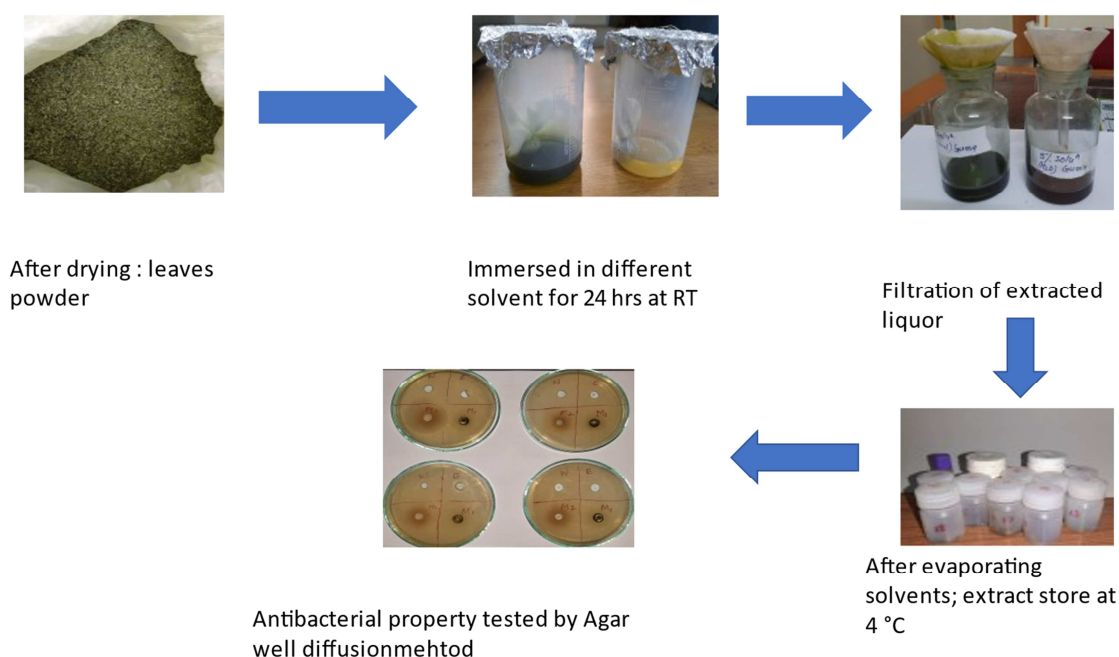


Figure 6. Flow chart for testing antibacterial property.

3.7. Determination of the Percentage Inhibition of Diameter Growth (PIDG)

The percentage inhibition of diameter growth (PIDG) values was evaluated according to the equation as below [20]:

$$\text{PIDG (\%)} = \frac{\text{Diameter of sample} - \text{Diameter of control}}{\text{Diameter of control}} \times 100$$

3.8. Determination of Phytochemical Compound

Presence of functional group is found qualitatively with this test. It reveals a broad idea about chemical group presence in crude extract of leaves powder [21, 23].

3.8.1. Test of Carbohydrates

(i). Fehling's Test

Equal volume of Fehling A and Fehling B reagents were

4.3. Antibacterial Efficiency Against Selected Plant Extract

Zone of inhibition (mm) and percentage inhibition of selected antimicrobial agents was calculated as shown in table 3.

Table 3. Zone of Inhibition in mm (ZoI for control=0.9mm).

Antibacterial Agents	Gram positive Bacteria- <i>S. aureus</i>			Gram negative Bacteria- <i>E. coli</i>		
	ZoI of Water Extract (mm)	ZoI of Ethanol Extract (mm)	ZoI of Methanol Extract (mm)	ZoI of Water Extract (mm)	ZoI of Ethanol Extract (mm)	ZoI of Methanol Extract (mm)
Guava	Nil	1.65	1.75	Nil	Nil	Nil
Parijaat	Nil	1.20	1.25	Nil	Nil	Nil
Merigold	Nil	1.45	1.56	Nil	Nil	Nil
Aakda	Nil	1.25	1.45	Nil	1.1	1.5
Durba	Nil	1.25	1.35	Nil	Nil	Nil

It was observed that among all five selected plants Guava leaves extract in ethanol and methanol has seen best antibacterial effect against gram positive microbes i.e. *S. aureus* while Aakda leaves extract in ethanol and methanol shows antibacterial effect against both microbes. Extraction study carried out in water does not exhibit any antibacterial effect because the phytochemical present in water extract is different from methanol and ethanol extract [24]. Also, polyphenol which were extracted from all selected antibacterial methanol and ethanol extract reveals no inhibition against *E. coli* microbes [25].

4.4. Percentage Inhibition of Diameter Growth (PIDG)

Table 4 shows PIDG for selected plant leaves. Maximum PIDG means good antibacterial effect. Guava leaves in methanol extract shows maximum antibacterial effect against *S. aureus* may be because maximum amount of presence of polyphenols, tannins and flavonoids. while aakada leaves shows antibacterial effect against both microbes as it is also having good UV protecting ability so good for making antibacterial bandage [22].

Table 4. Percentage Inhibition of Diameter Growth.

Antibacterial Agents	Gram positive Bacteria- <i>S. aureus</i>			Gram negative Bacteria- <i>E. coli</i>		
	PIDG Water Extract (mm)	PIDG Ethanol Extract (mm)	PIDG Methanol Extract (mm)	PIDG Water Extract (mm)	PIDG Ethanol Extract (mm)	PIDG Methanol Extract (mm)
Guava	Nil	83.33	87.62	Nil	Nil	Nil
Parijaat	Nil	33.33	38.88	Nil	Nil	Nil
Merigold	Nil	61.11	73.33	Nil	Nil	Nil
Aakda	Nil	38.88	61.11	Nil	22	66.66
Durba	Nil	38.88	50.0	Nil	Nil	Nil

5. Conclusion

Among the 5 selected plants; maximum antibacterial property were observed in methanol extract of Guava leaves against gram positive bacteria. As these bacteria are generally found in wound, consequently it is suitable for antibacterial wound dressing. While Aakda leaves extracted in water, ethanol and methanol shows antibacterial activity against both microbes but showing good antibacterial property in methanol extracted medium. Also aakda extracted agent shows good UV protection [22]. Hence among all 5 selected plant guava and aakda can be best for preparing wound dressing materials.

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