PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY OF BORASSUS FLABELLIFER L. LEAVES EXPOSED TO HUMAN PATHOGENS IN COLLECTED FROM DIFFERENT GEOGRAPHICAL AREA IN THOOTHUKUDI DISTRICT, TAMIL NADU

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Abstracts

The purpose of this study was to assess the phytochemical screening and antibacterial activity of Borassus flabellifer L. leaf extracts collected in the Thoothukudi district's Inland and Coastal areas against human pathogens. The antibacterial activity of B. flabellifer L. leaves was investigated using the agar well diffusion technique against a variety of test microorganisms, including bacteria and fungi. In the study on photochemistry, compounds such as flavonoids, alkaloids, phenol, protein, saponins, tannins, carbohydrates, and glycosides were investigated. Inland areas had the most (21 mm) and least (8 mm) zones of inhibition for E.coli and Staphylococcus albus, respectively. In coastal areas E.coli (21 mm) and Staphylococcus albus (9 mm) had the largest and smallest inhibition zones, respectively. B. flabellifer L. was found to have a higher rate of growth inhibition against some human pathogens. Ethanol and acetone extracts of B.flabellifer L. leaves have potent antibacterial properties and it could be used in the agro-food and pharmaceutical industries to create natural antioxidants.

Keywords: Leaf extracts, phytochemical screening, Antimicrobial activity, Inland and Coastal area, Borassus flabellifer L. Thoothukudi District

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1. Introduction

Borassus flabellifer L. is a member of the Arecaceae family and it is commonly known as the Palmyra palm. It is native to tropical Africa and is widely cultivated in India, Bangladesh, Burma, Sri Lanka, and Malaysia (Nesbitt, 2005; Jamkhande et al., 2016). Since the middle of the primitive time, the improvement and dissemination of knowledge about plants and their traditional therapeutic applications has been one of the foundations for the healing of general ailments (Awal et al., 1995; Greig, 1980). The Borassus aethiopum M. Borassus flabellifer L. and Borassus sundaicus B. are the three most economically important species of Borassus (Naguleshwaran, 2010). Palmyra palm leaves are utilized in various type of applications, including weaving, thatching, writing surfaces, umbrellas, hats, and basket (Sandhya et al., 2010). Furthermore, the Palmyra palm leaf midribs and stalk fibers are used to produce brushes, and the base of young leaf stalks is used to filter toddy and make fire lamp (Nadkarni, 2002). Palmyra plants are a good source of Antimicrobial compound and have a variety of therapeutic benefits (Peraman Muthu Kumaran, 2014). Gums, albuminoids, lipids, steroidal glycosides, and carbohydrates are found in Palmyra palm (Sandhya et al., 2010). The Palmyra palm is high in vitamins and minerals. The fruit's pulp can be used to treat skin disorders including dermatitis. According to sources, it contains anti-oxidant and anti-inflammatory properties. It can also be used to relieve nausea and vomiting. Toddy is a sugary sap generated by the immature male and female flowers of the plant. Fresh sap is high in B-complex vitamins. Furthermore, the sap can be used to treat ulcers and liver diseases (Morton, 1988). The sap from the Palmyra palm's flowering portion has been used as a diabetic patient's sweetener and in traditional recipe (Masayuki and Fengming, 2007). The root extract of Borassus flabellifer L. has been found to have antibacterial properties (Vedha Priya et al., 2016).

Moreover, the fruits and root tubers of Palmyra palm are abundant in a number of nutrients including sugar, calcium, iron and polyphenols (Paschapur et al., 2009). Biological activity and pharmacological functions have been described in several portions of the Borassus flabellifer L. including anthelmintic, diuretic, antioxidant, antibacterial, wound healing, immunomodulatory (Rios, 2010) and antimalarial properties (Pramod et al., 2013; Pattanaik et al., 2008 and Koudouvo et al., 2011). Many studies have demonstrated that synthetic antioxidants have negative consequences such as carcinogenesis and liver damage, both directly and indirectly (Li et al., 2012). Alkaloids, tannins, flavonoids, and phenolic compounds are the most significant phytochemicals found in Palmyra palm leaves (Iwu, 2000). Microbial infection is one of the most frequent causes of oxidative responses, which finally result in cell damage (Sandhya et al., 2010). As a result, the current study was conducted to investigate the quality and antimicrobial activity of Borassus flabellifer L. leaf extract collected from the inland and coastal areas of Thoothukudi district.

2. Materials and Methods

2.1 Plant material collection

In Inland areas, Borassus flabellifer L. leaves were collected from Kottarakurichi village of Eral taluk (KK-8.63 46' N and 78.02 67' E) and in Coastal areas, Adaikalapuram village of Tiruchendur taluk (AP-8.54 41' N and 78.10 26' E) in

Thoothukudi District, Tamil Nadu. These taluks are 27 and 38 kilometres north and south of Thoothukudi district, respectively. The taxonomic identity of plant material was validated by the 'Flora of the Presidency of Madras' (Gamble, 1928) and the 'Flora of the Tamil Nadu Carnatic' (Mathew, 1981).

2.2 Phytochemical Analysis

Solvent Extraction

The mature and healthy plant was collected, thoroughly washed and transformed into small fragments in a hot air oven at 72°C for 4 days. A grinder was used to finely powder the Palmyra leaves (rough powder by sieve No. 250 m, manual). The samples were sealed in a ziplock bag and stored at room temperature. About 30 g of plant powder was placed in a digestion flask attached to a Soxhlet apparatus, and extracts were obtained using acetone, benzene, distilled water, petroleum ether, and ethanol. The aqueous extract was made by boiling the powder in distilled water directly. Solvents were chosen based on phytochemical solubility and polarity. The solvent was evaporated, and the residue was

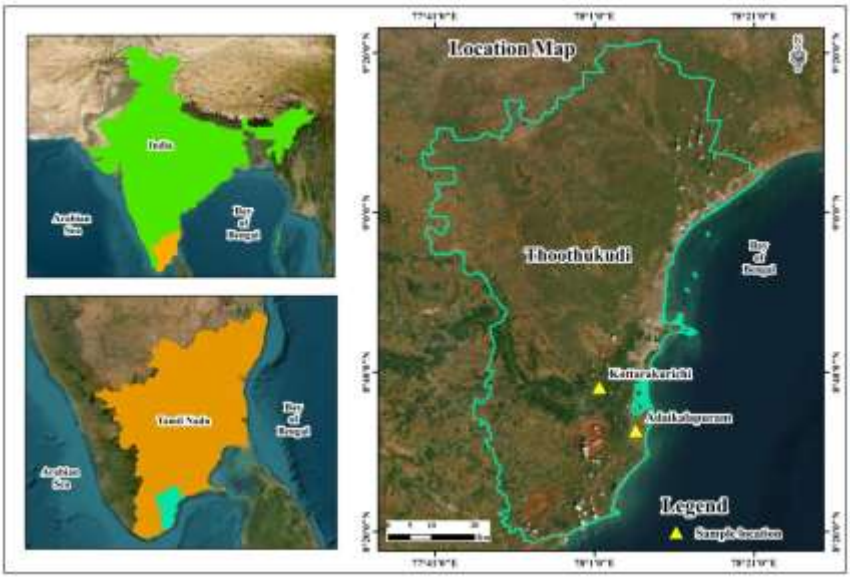

Figure 1: Map showing the location of 2 tropical and sub-tropical regions in (Inland and Coastal sites)
Thoothukudi District of Tamil Nadu, India (ArcGIS Version-10.8)

Figure 2: Natural Habitat of Borassus flabellifer L. and mature leaves

dissolved in 50 mg/ml sterile Dimethylsulfoxide (DMSO-9:1). The extract was filtered using 2 mL microcentrifuge tubes (Type: Eppendorf safe-lock) and kept at 4°C for further analysis.

2.3 Screening for Preliminary Phytochemicals

The phytochemical constituets of Borassus flabelliefer L. solvent extracts were assessed qualitatively using standard techniques published by Haborne, 1991 and Sofowora, 1993. The alkaline solvent experiment had been used to determine Flavonoids; the lead acetate experiment was used to identify Phenol (Chandra mohan et al., 2012). The Mayer's test was used to determine Alkaloids (Evans, 2002). Protein was determined using the Biuret test (Harborne, 1998). The tannins were ascertained using the Ferric chloride test (Hossein Mostafavi et al., 2015). Saponins were identified using the foam test (Gothandam et al., 2010). The Molish's test was used to detect carbohydrate (Bandiola et al., 2017) and legal's test was used to identify glycosides (Siddiqui and Ali, 1997). Preliminary phytochemical screening was carried out to study the various types of natural chemicals found in the extract.

2.4 Screening for Antimicrobial Activity

Using the Agar well diffusion method, the efficacy of leaf extracts of Borassus flabellifer L. was tested against the growth of a few human pathogens in a clinical laboratory (Scudder Diagnostic Centre, Nagercoil). The Kirby-Bauer method was used to test the antibacterial activity of isolated plant extraction pellets (Bauer et al., 1996). The overnight grown cultures of respective bacteria and fungi were marked on the Nutrient agar, Sabourauds Dextrose Agar (SDA), and Potato Dextrose Agar (PDA). A good puncher was used to make wells in all of the plates and in each of the plates wells were also made for the controls. At a concentration of 50 mg/mL, leaf extracts (acetone, benzene, distilled water, Petroleum ether, and ethanol) were prepared. Antibiotics (Amikacin and Nystatin) at a

concentration of 50 mg/mL were used as controls. The bacteria (E.coli, Klebsiella pneumoniae, Bacillus substills, and Stephylococcus epidermis) plates were also incubated at 37°C for 24 hours, while the fungi (Aspergillus niger, Candida albicans) plates were incubated at 35°C for 48 hours. The diameter of the inhibition zone was measured in millimetres (Peraman Muthu Kumaran, 2014).

3. Results and Discussion

3.1 Screening for phytochemicals

Inland areas

A phytochemical study was used to evaluate the qualitative analysis of Borassus flabellifer L. leaf extracts in Inland areas including flavonoids, alkaloids, phenol, protein, saponins, tannins, carbohydrates, and glycosides. Among all the solvents, acetone and benzene extracts confirmed the presence of alkaloids, phenol, proteins, tannins, carbohydrates, and glycosides. Flavonoids, saponins, and glycosides were discovered in distilled water extracts. Furthermore, alkaloids, tannins, carbohydrates, and glycosides were identified in petroleum ether and ethanol extracts from inland areas, which may involve in exhibiting antibacterial activity (Table 1).

Table 1: Phytochemical components of Borassus flabellifer L. leaves in Inland areas

Phytochemical constituents	**Acetone**	**Benzene**	**Water**	**Petroleum ether**	**Ethanol**
Flavonoids	-	-	+	-	-
Alkaloids	-	+	-	+	-
Phenol	-	+	-	-	-
Proteins	-	+	-	-	-
Tannins	+	-	-	+	+
Saponins	-	-	+	-	-
Carbohydrates	-	+	-	+	-
Glycosides	-	+	+	+	+

(+) Presence of the phytochemical constituents; (-) Absence of the phytochemical constituents

Coastal areas

Alkaloids, phenol, proteins, tannins, and carbohydrates were found in acetone and benzene extracts of leaves collected in coastal areas. The distilled water extract contained flavonoids, tannins, saponins, and glycosides. Furthermore, compounds such as phenol, tannins, carbohydrates, and glycosides were found in the petroleum ether and ethanol extracts (Table 2).

Table 2: Phytochemical components of Borassus flabellifer L. leaves in Coastal areas

Phytochemical constituents	**Acetone**	**Benzene**	**Dis. H_2O**	**Petroleum ether**	**Ethanol**

Flavonoids	-	-	+	-	-
Alkaloids	-	+	-	-	-
Phenol	-	+	-	+	-
Proteins	-	+	-	-	-
Tannins	+	-	+	+	+
Saponins	-	-	+	-	-
Carbohydrates	-	+	-	+	-
Glycosides	-	-	+	+	+

(+) Presence of the phytochemical constituents; (-) Absence of the phytochemical constituents

Borassus flabellifer L. is the most extensively used ancient herbal remedies (Pramod et al., 2013; Keerthi et al., 2007). Several studies have shown that extracts and fractions generated from various phytochemicals can prevent growth of bacteria (Alabri et al., 2014). The extracts and fractions of antimicrobial effectiveness against clinical pathogenic strains of bacteria and fungus verified the plant's historical use as a remedy (Tankeo et al., 2015).

The present study performed preliminary phytochemical screening procedures of Borassus flabellifer L. leaf extracts. The analysis indicates the existence of alkaloids, flavonoids, tannins, phenol, protein, saponins, carbohydrates, and glycosides. Similarly, terpenoids, steroids, and saponins were found in Borassus aethiopum extracts, according to a phytochemical investigation. All of these compounds have been demonstrated to be effective antioxidants. (Chanwitheesuk et al., 2005; Hafidh et al., 2009; Rashmi et al., 2011 and Roy et al., 2011). Alkaloids are one of the most significant groups of phytochemicals that have resulted in the formation of therapeutic analgesic. These have been employed in treatment particularly the steroidal alkaloids, which have a protective effect in animals (Kam and Liew, 2002; Lata and Dubey, 2010). Flavonoids and tannins are phenolic substances that function as natural antioxidants have antimicrobial and anti-inflammatory, anti-allergic, anti-neoplastic anti-inflammation characteristics and are used to treat intestinal illnesses. Tannin-rich plant formulations have been utilized in Traditional medication to cure disorders such as leucorrhoea, rhinorrhea, and constipation (Rievere et al., 2009; Doughari, 2012).

Furthermore, saponins are commonly assumed to be antinutrients, although they are also known to be advantageous in human nutrition for cholesterol control. As a result, the existence of the pulp powder may signal that it has medicinal effect (Frankel, 1995). Polymeric phenolic compounds are particularly significant cellular support substances since they are an inherent component of the cell wall structure (Gupta et al., 2010). Bioactive polyphenols have received a lot of interest, since they can protect the human body against oxidative stress, which may lead to a range of diseases like cancers, cardiac issues, and ageing (Robards et al., 1999). Glycosides have been used to treat hypertension for a long time (Watt and Breyer-Brandwyk, 1984). As a consequence, the findings of this study show that the chemical compounds identified could be pharmaceutical substances.

3.2 Antibacterial Activity assay

The antibacterial activity of Borassus flabellifer L. leaf extracts such as Acetone, Benzene, Distilled water, Petroleum ether, and Ethanol showed good activity when

measured by the diameter zone of inhibition against four human pathogens, Bacillus Substils, Staphylococcus and albus (Gram positive bacteria), E.coli and Klebsiella pneumonia (Gram negative bacteria) (Fig.6 and 7)

Inland area

Inland areas had the largest and least zone of inhibition against ethanolic extracts of E.coli (21 mm) and distilled water extracts of staphylococcus albus (8 mm) respectively (Fig. 4).

Coastal area

In coastal areas, the ethanolic extracts of E.coli (21 mm) and distilled water extracts of Staphylococcus albus (9 mm) had the largest and smallest zones of inhibition respectively (Fig. 5).

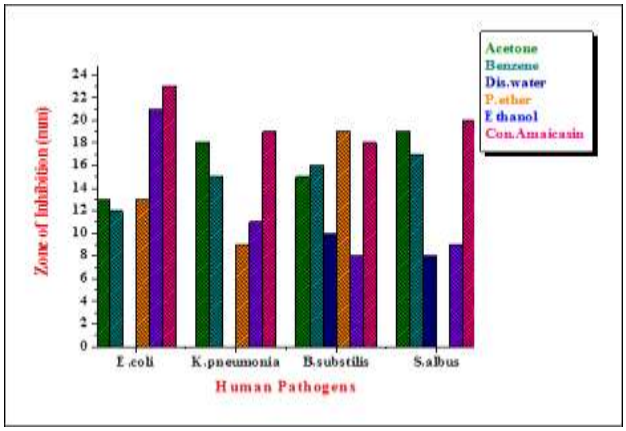

Fig. 4: Antibacterial activity of leaf extract of Borassus flabellifer L. (Inland area) (Origin-Version 6.0).

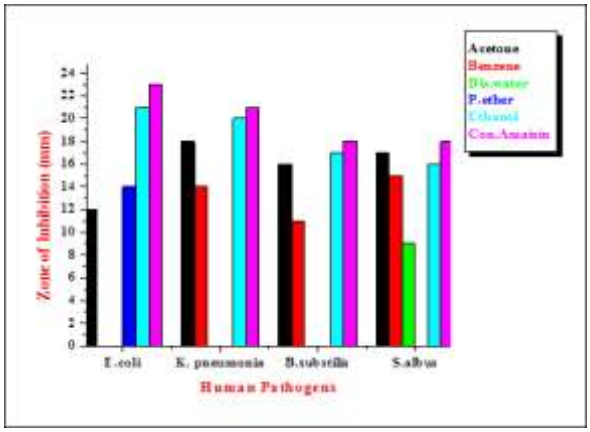

Fig. 5: Antibacterial activity of leaf extract of Borassus flabellifer L. (Coastal area) (Origin-Version 6.0).

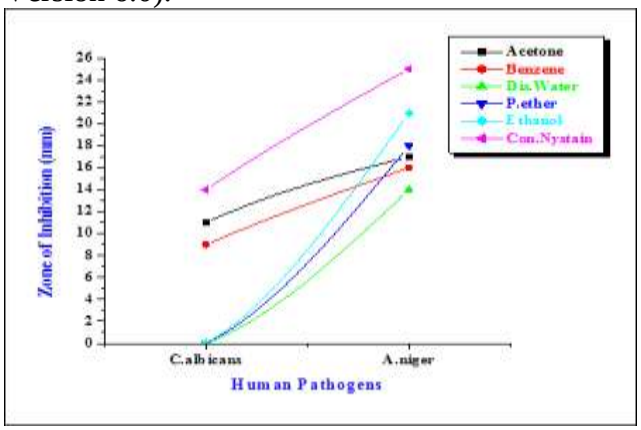

Figure 8: Antifungal activity of leaf extract of Borassus flabellifer L. (Inland area) (Origin-Version 6.0).

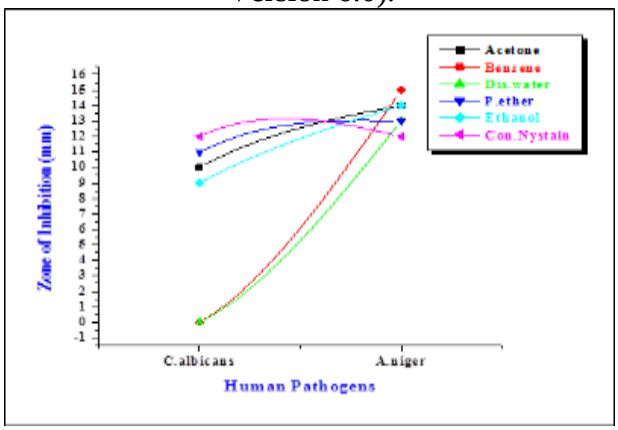

Figure 9: Antifungal activity of leaf extract of Borassus flabellifer L. (Coastal area) (Origin-Version 6.0).

3.3 Antifungal activity assay

Antifungal activity of Borassus flabellifer L. leaf extracts were tested for antifungal activity against two strains of Aspergillus niger and Candida albicans. The antifungal activity of various extract of B. flabellifer L. leaves has been confirmed (Fig. 7).

Inland area

The maximum and minimum number of zones of inhibition against Candida albicans of acetone (11 mm) and benzene (9 mm) extracts were observed in inland areas, respectively. Ethanol (21 mm) and distilled water (14 mm) extract had the largest and smallest zones of inhibition was exhibited against Aspergillus niger (Fig. 8).

Coastal area

The largest and least number of zones of inhibition was exhibited against Candida albicans of petroleum ether (11mm) and ethanol (9 mm) extracts were seen in Coastal areas, respectively. Benzene (15 mm) and distilled water extract (13 mm) had the biggest and smallest zones of inhibition were showed against Aspergillus niger, respectively (Fig. 9).

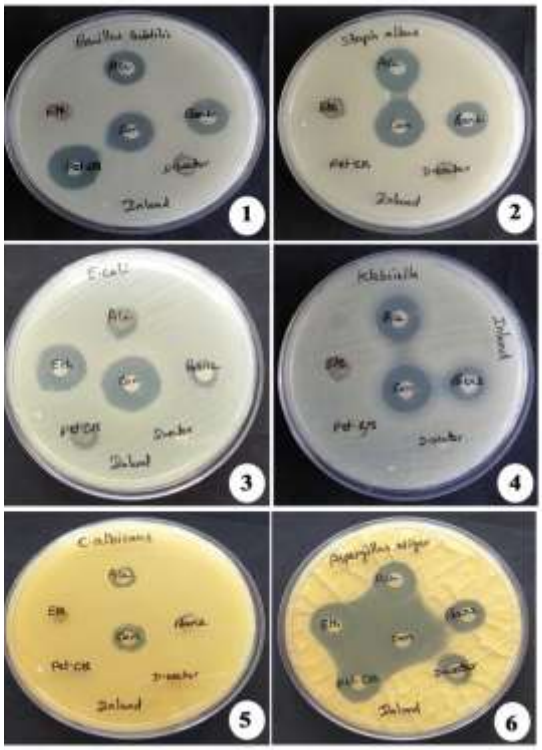

Figure 6: Antimicrobial activity of Borassus flabellifer L. leaf extracts using a disc diffusion assay in the Inland area 1. Bacillus Substils
2. Staphylococcus albus 3. E.coli 4. Klebsiella pneumonia phenomeno 5. Candida albicans
6. Aspergillus niger

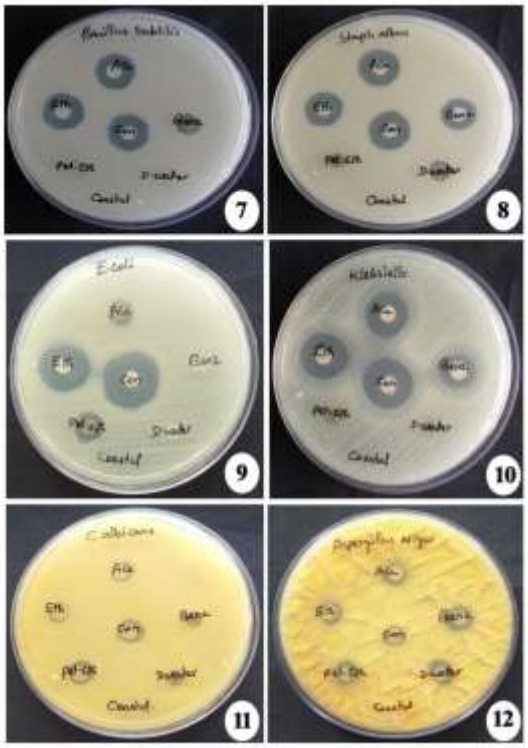

Figure 7: Antimicrobial activity of Borassus flabellifer L. leaf extracts using a disc diffusion assay in the Coastal area 7. Bacillus Substils
8. Staphylococcus albus 9. E.coli 10. Klebsiella pneumonia phenomeno 11. Candida albicans
12. Aspergillus niger

The standard antimicrobial Amaikacin and Nystain have been used to compare Borassus flabellifer L. leaf extracts including both inland and coastal areas. In antimicrobial investigations, the ethanol extract of B. flabellifer L. leaf exhibited the largest zone of inhibition against A.niger and E.coli with diameters of 21mm and 21mm, accordingly, in inland regions. In coastal areas, the ethanol extract of B. flabellifer L. leaf demonstrated the highest zone of inhibition against A.niger and E.coli, with diameters of 21mm and 14 mm, respectively. According to previous study on the antibacterial activity of B.flabellifer L. root extracts in ethanol and methanol, the maximum zone of inhibition against 14 mm (Saravanan et al., 2012).In comparison to the other solvents tested, ethanol extracts of B.flabellifer L. leaves were more effective and demonstrated strong antibacterial activity against all microorganisms investigated. The distilled water extracts had the most limited antimicrobial action against all pathogens 8-14 mm, whereas the acetone and Petroleum ether extracts showed moderate inhibition to the pathogens 11-19 mm in the Inland and Coastal areas, respectively. According to the findings, ethanol solution reported to inhibit various bacteria in different zones. The zone of inhibition was greatest in the dry peel endocarp (young) extraction of Borassus flabellifer L. Bacillus subtilis exhibited the greatest level of inhibition against ethanol extract 45 mm, whereas Pseudomonas aeruginosa and Bacillus subtilis exhibited the greatest zone of inhibition in ethanol extract 39 mm. However, as compared to antibiotics, it has a higher level of action. The Pseudomonas aeruginosa is inhibited by acetone extracts to the greatest extent possible (Lakshmanan et al., 2022).

The Acetone and Petroleum ether extracts is shown to be moderately effective against the test organisms. However, as comparison to the benzene and distilled water extracts, the ethanolic extract demonstrated a significant increase in antimicrobial activity. This might be owing to the presence of more antimicrobial compounds (phytochemicals) in the ethanolic extract. As a consequence, one of the most efficient solvents for phytochemical extraction is ethanol. The greater zone diameter was caused by the greater concentration of extracts (50mg/mL) employed in this study. According to the findings of this study, as the concentration of the extracts grows, so does their antimicrobial activity.

4. Conclusion

The current study mainly focused on two features of the Palmyra palm. In the first part of the presence or absence of chemical compounds in Palmyra palm tree leaf extracts was evaluated. The antimicrobial activity of Borassus flabellifer L. leaves was investigated of in the second part of the study and it was found that ethanol and acetone extracts exhibit strong antibacterial activity against the majority of the bacterial and fungal strains evaluated. The Borassus flabellifer L. leaves have been shown to be high in natural antioxidants and antimicrobials, suggesting that they might have a wide range of uses in agro-food and pharmaceutical industries.

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