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Antibacterial and antifungal activities from leaf and bark extract of *Cassine glauca* (Rottb.) Kuntze

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ABSTRACT

Practical knowledge of traditional medicine from ancient times, medicinal plants, often known as medicinal herbs, have an extensive history. Plants produce hundreds of different kinds of chemicals to serve various purposes, such as defense and protection against pathogens, fungi, insects, and herbivorous mammals. The present study investigates the antibacterial and antifungal potential of *Cassine glauca* leaves and bark extract. The antimicrobial activity was to find out in the extracts by using the agar disc diffusion method. Chloroform, ethanol and ethyl acetate extracts were obtained from the leaves and bark. It showed that antimicrobial activity against tested microbes from the four bacterial strains such as *Staphylococcus aureus* (MTCC 9542), *E. coli* (MTCC 732), *Klebsiella pneumonia* (MTCC 3040) and *Proteus vulgaris* (MTCC 7299) and three fungal strains such as *Aspergillus niger* (MTCC 1785), *Aspergillus versicolor* (MTCC 280) and *Candida albicans* (MTCC3958). The zone of inhibition of leaf and bark extracts were compared with standards like Ampicillin, Methicillin, and Norfloxacin for antibacterial and Terbinafine and nystatin for antifungal activity. The antimicrobial study result reveals that good inhibition of the bacterial and fungal grown against the tested microbes. The antimicrobial activity showed that to justify the use of their plant in traditional medicine and the practice of supplementary decoction.

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INTRODUCTION

India is one of the top most nations using medicinal and herbal plant resources for medicinal purposes to treat various diseases. The traditional and folk peoples are used herbal medicines. Studies using ethno botanical folklore and ethno veterinary methods were used to identify most therapeutic plants. Eighty percent of the world's population gets their pharmaceuticals and drugs from medicinal plants, according to research by the World Health Organization (Samoisy & Mahomoodally, 2015). Over the world, plants have been used to treat a wide range of illnesses in both human beings and animals. They also include chemical components utilized to create new medications (Sofowora, 1982). With the emergence of immunological resistance, it is a practical and green product (Githiori *et al.*, 2006).

The antibacterial and wound-healing properties of a few medicinal herbs were investigated by Moglad *et al.* (2020). Drug-resistant bacteria are emerging more frequently every day. The evaluation of the medicinal plant extracts and its antibacterial

properties is the goal of several investigations. It said that there are few documented instances of the medicinal plant *C. glauca* treating wounds. According to Doughari *et al.* (2007), Pirbalouti *et al.* (2009) and Sapkota *et al.* (2012), food poisoning is regarded as a frequent cause of disease globally. The majority of food poisoning cases are linked to bacterial contamination, particularly the Gram-positive bacteria *Staphylococcus aureus*, which has also been linked to food borne illnesses and food spoilage (Braga *et al.*, 2005). Plant extracts are recognized as natural resources of antimicrobial agents that are safe for human nutrition and readily biodegradable (Cowan, 1999; Duffy & Power, 2001; Berahou *et al.*, 2007; Ogbulie *et al.*, 2007).

Medicinal plants have long been employed in traditional medicinal systems and are a valuable natural resource that humans can utilize to achieve improved health by providing sources of bioactive chemicals from nature (Moglad *et al.*, 2020). Antibiotics play the most significant role in fighting against infectious bacteria and fungi. Nowadays, many antimicrobial strains produce toxic substances and it's an urge to develop new drugs from medicinal plants. WHO reported that several

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herbal plant species and their active phytochemicals are used in traditional medicine system.

Many chemical substances that are produced by medicinal plants seem to play a direct role in the development and proliferation of secondary metabolites. These secondary metabolites are not the same as the primary metabolites of plants, which include simple carbohydrates, amino acids and nucleotides and other constituents etc. Three chemical classes comprise the secondary metabolites like phenolics, alkaloids, and terpenes. The latter group includes secondary products that contain nitrogen. The capacity of plants to produce these chemicals has been linked to their biological characteristics and applications. One of the biologically significant characteristics of essential oils is their antimicrobial activity, which is often attributed to the presence of active monoterpene constituents. These compounds can either directly inhibit enzymes or form complexes with specific enzymes (Kalemba & Kunicka, 2003; Duarte *et al.*, 2016). *C. glauca* belongs to the family Celastraceae. Its natural habitat is found in subtropical mixed deciduous and evergreen forests throughout the Indo-Malayan regions. It is extremely prevalent in Sri Lanka and the foothills up to 1200 meters.

The synthesis of new, therapeutically effective antibacterial medicines depends heavily on phytochemicals and its related molecules (Strohl, 2000). Numerous people utilize the *C. glauca* plant species as cow fodder, and only a small number of people use it as medicine. This is especially true for the deep forest inhabitants of India, Sri Lanka, Indonesia, and Africa (Liu *et al.*, 2008). *C. glauca* is commonly used against snake bites, headache, wound healing in animals, antidote stimulant, cold and antirheumatic in traditional ethnoveterinary medicine system.

The discovery of novel herbal medications for the management of diverse illnesses in humans. It is still highly challenging for scientists to determine which phytocompounds in medicine are active as well as for the pharmaceutical industry. The use and importance of herbal medicines raise their worth in the information market in terms of their acknowledgement and contribution to the human health care system. The *C. glauca*, which grows throughout the Western Ghats, is used by the Thalavumalai peoples in the Erode district of Tamil Nadu State. The present research were carried out, to screen the leaves and bark extracts of *C. glauca* against pathogenic bacterial and fungal strains and to identify new source of antimicrobial agents.

MATERIALS AND METHODS

Collection of Samples

Leaf and bark of *Cassine glauca* (Rottb.) Kuntze were collected in Namakkal, and identified with the help of a plant taxonomist and voucher specimen was deposited in the herbarium (#NKL 2019/12) of PG & Research and Department of Botany, Arignar Anna Government Arts College, Namakkal.

The collected plant samples were dehydrated in a shaded area. The bark and leaves were ground into a coarse powder after

optimal drying and they were kept apart in a tightly sealed container for upcoming laboratory testing.

Preparation of Solvent Extracts

Different types of solvents such as chloroform, ethanol and ethyl acetate were used to prepare crude extracts for the present study. Fine powdered plant samples (20 gm) were packed into the thimble of the extraction apparatus, which was placed in the Soxhlet tube and subjected to an 8-hour extraction process using heat. The concentrated vapors obtained from the extracts were further processed and dehydrated using a Rotary Vacuum Evaporator (Equitron, India) at a temperature of 60 °C under reduced pressure. The solvent was carefully evaporated, and the resulting dried crude residues were aseptically weighed, the extract value was then calculated using the formula: Weight of the dried extract/Weight of the plant material × 100. The extracts were then re-dissolved in their respective solvents and stored at 4 °C in sterile, labeled, airtight containers for subsequent analysis. This meticulous process ensured the preservation and concentration of the plant extracts for subsequent investigations (Sreelatha & Padma, 2009).

Nutrient Broth Preparation

Twenty mL of broth culture media, peptone - 0.1 g, sodium chloride - 0.16 g, beef extract 0.06 g, yeast extract 0.06 g, and distilled water 20 mL were mixed and sterilized for 45 minutes, used for fungal culture and broth culture media was used for bacterial culture.

Antifungal Activity

The Mueller Hinton agar medium was used to test the antibacterial activity of solvent and Chloroform extracts by using the disc diffusion method (Satish *et al.*, 1999). Mueller Hinton agar medium agar plates were used to create discs using sterile corpora (5 mm), and solid plates were inoculated with bacteria using sterile swales wet with the bacterial suspension. Subsequently, 50 mL of every chloroform and solvent extract were added to the disc that was created in the inoculated plates. This antibacterial activity was achieved by the well-spread technique using a laminar airflow chamber. The well-spread approach makes use of Mueller Hilton agar media. Regarding the analysis of the plant extract of *C. glauca* for antibacterial activity, an agar disc diffusion method was used to test bacterial strains. The bacterial strains obtained from the Institute of Microbial Technology, Chandigarh were used for the current study. Including bacterial strains such as *E. Coli* (MTCC 732), *Staphylococcus aureus* (MTCC 9542), *Proteus vulgaris* (MTCC 7299) and *Klebsiella pneumonia* (MTCC3040) and three fungal strains such as *Aspergillus niger* (MTCC 282), *Aspergillus clavatus* (MTCC 1323) and *Candida albicans* (MTCC227) were selected based on pharmacological significance. Both the bacterial and fungal stocks are generally incubated at 37°C for one day on PDA and nutrient agar medium. The stock cultures were stored and maintained in the refrigerator at 4 °C.

Zone of Inhibition Determination

Both the antibacterial and antifungal activities were evaluated for chloroform, ethanol and ethyl acetate. Four antibacterial strains and three antifungal strains response against plant leaves and bark extracts. Both leaves and bark extracts were generally dissolved in dimethyl sulfoxide, filtered and stored at 4 °C. The zone of inhibition determination was evaluated antibacterial and antifungal cultures with standard antibiotics. The results were compared with microbial strains and antibiotics. The concentration of leaves and bark extracts (5, 25, 50, 75 and 100 µg/mL) of *C. glauca*, standard drugs were thoroughly dissolved in double distilled water by utilizing nutrient agar tubes. Control experiments were utilized in same condition with the help of Ampicillin, Methicillin, Norfloxacin for antibacterial and for Terbinafine and Nystatin antifungal as good drugs as standard one. The zone of inhibition was measured around the disc after 24 hrs at 37 °C incubation and 50 to 96 hrs for fungal at 28 °C incubation.

RESULTS

The antimicrobial activity studies were carried out the leaves and bark extracts of *C. glauca* in various concentrations (5, 25, 50, 75 and 100 µg/mL) against four different bacterial pathogenic strains such as *Staphylococcus aureus*, *Klebsiella pneumonia*, *E. coli* and *Proteus vulgaris* also three fungal pathogenic strains such as *Aspergillus niger*, *Candida albicans* and *Aspergillus versicolor*. These selected strains on the basis of its application and formulation investigation for analysis.

Antibacterial and antifungal efficacy of leaves and bark extracts were analysed in zone of inhibition of growth rate of bacteria and fungal strains. The results are shown in Tables 1 - 6.

Three solvent extracts chloroform, ethanol, and ethyl acetate were reportedly utilized most frequently. The antibacterial activity of Gram-positive and Gram-negative bacterial strains was examined in three different solvent classes. The high-polar solvent is water, the mid-polar solvent is ethanol, and the low-polar solvent is ethyl acetate. The majority of the ethyl acetate extract's low polarity makes the *C. glauca* bark sample susceptible to these bacteria, and the leaf is equally susceptible.

In the present study, antibacterial and antifungal efficacy of the leaves and bark extracts were increasing trend when increasing the concentration of both leaves and bark extracts of the *C. glauca* in µg/mL when compared with the standard drug the results showed that more sensitive bark than the leaves. The results are expressed that *C. glauca* were found to be more efficient against all the tested bacterial and fungal strains.

DISCUSSION

According to Yang *et al.* (2001), phenolic compounds in food should be viewed as possible antioxidants rather than carcinogens. According to Hedlund *et al.* (2003), phenolics influence the proliferation of both malignant and benign

Table 1: Antibacterial activities of leaf extracts of *C. glauca* against bacterial strains

S. No. Bacterial strains		Zone of inhibition (mm)														
		Concentration (µg/mL)					Concentration (µg/mL)					Concentration (µg/mL)				
		Chloroform					Ethanol					Ethyl acetate				
		5	25	50	75	100	5	25	50	75	100	5	25	50	75	100
1	<i>Staphylococcus aureus</i>	-	-	8	11	17	-	5	8	13	19	-	7	9	12	18
2	<i>E. coli</i>	-	6	8	10	18	-	-	10	12	18	-	-	10	12	19
3	<i>Klebsiella pneumonia</i>	-	7	10	13	17	-	6	10	14	18	-	7	9	11	19
4	<i>Proteus vulgaris</i>	-	-	11	14	16	-	-	10	12	17	-	-	10	13	17

Table 2: Antibacterial activities of bark extracts of *C. glauca* against bacterial strains

S. No.	Bacterial No. strains	Zone of inhibition (mm)														
		Concentration (µg/mL)					Concentration (µg/mL)					Concentration (µg/mL)				
		Chloroform					Ethanol					Ethyl acetate				
		5	25	50	75	100	5	25	50	75	100	5	25	50	75	100
1	<i>Staphylococcus aureus</i>	-	6	9	12	18	-	6	7	12	18	-	6	7	11	17
2	<i>E. coli</i>	-	6	8	11	18	-	6	9	13	17	-	6	8	12	18
3	<i>Klebsiella pneumonia</i>	-	6	9	12	17	-	6	8	12	19	-	6	9	13	20
4	<i>Proteus vulgaris</i>	-	6	8	11	17	-	6	7	12	18	-	6	7	13	11

Table 3: Antibacterial activity of standard drugs against bacterial strains

Drug	Concentration (µg/mL)	Zone of inhibition (mm)			
		<i>Staphylococcus aureus</i>	<i>E. coli</i>	<i>Klebsiella pneumonia</i>	<i>Proteus vulgaris</i>
Ampicillin	5	12	12	10	11
	25	14	13	14	15
	50	16	17	16	17
	75	20	21	22	21
	100	24	25	24	24
Methicillin	5	13	12	11	12
	25	15	16	17	18
	50	16	17	18	21
	75	22	23	22	24
	100	28	29	28	27
Norfloxacin	5	10	11	10	11
	25	15	16	15	16
	50	19	20	22	24
	75	23	24	26	26
	100	29	29	29	29

Table 4: Antifungal activities of leaf extracts of *C. glauca* against fungal strains

S. No.	Fungal strains	Zone of inhibition (mm)														
		Concentration (µg/mL)					Concentration (µg/mL)					Concentration (µg/mL)				
		Chloroform					Ethanol					Ethyl acetate				
		5	25	50	75	100	5	25	50	75	100	5	25	50	75	100
1	<i>Aspergillus niger</i>	-	10	12	16	19	-	9	12	14	20	-	8	10	13	17
2	<i>Aspergillus versicolor</i>	-	9	11	15	19	-	9	11	15	19	-	7	11	14	16
3	<i>Candida albicans</i>	-	10	13	16	18	-	10	12	14	19	-	8	11	13	18

Table 5: Antifungal activities of bark extracts of *C. glauca* against fungal strains

S. No.	Fungal strains	Zone of inhibition (mm)														
		Concentration (µg/mL) Chloroform					Concentration (µg/mL) Ethanol					Concentration (µg/mL) Ethyl acetate				
		5	25	50	75	100	5	25	50	75	100	5	25	50	75	100
1	<i>Aspergillus niger</i>	-	9	13	15	18	-	10	12	14	18	-	7	11	13	18
2	<i>Aspergillus versicolor</i>	-	10	12	16	19	-	9	11	14	20	-	8	10	14	19
3	<i>Candida albicans</i>	-	9	11	14	17	-	10	12	15	19	-	7	10	13	17

Table 6: Antifungal activity of standard drugs against fungal strains

Drug	Concentration (µg/mL)	Zone of inhibition (mm)		
		<i>Aspergillus niger</i>	<i>Aspergillus versicolor</i>	<i>Candida albicans</i>
Terbinafine	5	18	17	15
	25	24	23	22
	50	26	26	26
	75	28	28	28
	100	30	29	29
Nystatin	5	17	19	15
	25	21	19	18
	50	23	22	21
	75	26	25	24
	100	29	29	27

epithelial cells in culture. Antifungal (Zammit *et al.*, 2014; Parveen *et al.*, 2016), antibacterial (Mathur & Kamal, 2012), and antiparasitic (Das *et al.*, 2014) properties were demonstrated by phytochemicals and their derivatives. Singh *et al.* (2025) reported that green synthesis of silver nanoparticles by using neem plant extract and observed antimicrobial activity.

Medicinal plant extract and its derivatives are utilized for a variety of pharmacological activities, according to (Sun *et al.*, 2006). To treat illnesses, phytochemicals are extracted from a variety of plant resources (Venkatachalam & Jebasam, 2010). Phytochemical elements present in medicinal plant extracts are highly valuable in their fight against infectious pathogenic organisms. Based on current research, plant extract has good antibacterial activity against both Gram-positive and Gram-negative bacteria. Numerous bioactive components of phytochemicals include tannins, flavonoids, alkaloids, phenolic compounds, and saponins reported by Karar and Kuhnert (2017) and Moglad *et al.* (2020). Adem *et al.* (2024) stated that to find out phytochemicals and its antimicrobial activity of *Ficifolius* root extract.

The phytochemical components of this medicinal plant have the potential to be very beneficial in the battle against infection and bacterial destruction. The bacteria *C. glauca* used against in this study include *P. vulgaris*, *S. aureus*, *E. coli*, and *K. pneumonia*. For this antibacterial action, the bark and leaves of the plant samples are utilized. In comparison to all testing bacteria, bark samples exhibit significantly higher levels of antibacterial activity compared to leaf samples. Most antibacterial activity is seen in *K. pneumonia*. The green synthesis of silver nanoparticles by utilizing herbal medicinal plants *Swertia chirata* and identified as good antimicrobial properties (Shereen *et al.*, 2024).

The *C. glauca* plant in this study demonstrated the highest level of antibacterial activity against both Gram-positive and Gram-negative bacteria, and its performed exceptionally well in terms of healing wounds and eliminating bacterial stains. Extracts from the leaves and bark of *C. glauca* have not been shown to have any wound-healing properties. The *C. glauca* plant has been shown in several researches to be effective against a variety of bacteria (Elegami *et al.*, 2001).

Numerous studies have indicated the potential antibacterial properties of medicinal plants against bacteria, and our research has nearly reached this conclusion (Elegami *et al.*, 2001). Mamarasulov *et al.* (2023) is a good medicinal and endemic plant species, commonly used in traditional medicinal system and its also also active antimicrobial properties. These are the conclusions drawn from several scientific research papers Verma *et al.* (2012) and Qader *et al.* (2013) that examined the effectiveness of plant extracts and their active ingredients as antimicrobials to inhibit the growth of bacterial pathogenic organisms (Sheikh *et al.*, 2010). The research was conducted by Gupta and Vyas (2021) on the antibacterial activity of the cyclic peptide Nostophycin, which was isolated from wastewater Cyanobacteria.

Cassia stem has been shown to have antimicrobial activity reported by Chavan *et al.* (2011). Previous studies have shown that this stem's ability to inhibit plant extracts' hydrophobicity features and allow them to react with the microbial cell membrane and mitochondrial protein, disrupting their structure and converting to permeability (Friedman *et al.*, 2004; Tiwari *et al.*, 2009).

CONCLUSION

Most of the chosen microbes impact our plant parts, specifically the leaves and bark, according to our antimicrobial investigation. A variety of beneficial bioactive chemical substances are found in *C. glauca*, a plant that is not only utilized in traditional medicinal systems. The research on antimicrobial agents takes into account plant leaf and bark samples that are harmful to bacterial and fungal pathogens. Toxicology and pharmacological research on the plant are needed to be evaluated.

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