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Antimicrobial activity of Bark Extracts of *Elaeodendron paniculatum* Wight and Arn.

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The *in vitro* bioassay of the stem bark extract of *Elaeodendron paniculatum* was done against five bacterial strains- *Bacillus subtilis*, *Escherichia coli*, *Micrococcus flavus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* and one fungal strain *i.e., Aspergillus niger*. Acetone and ethanol extract showed good activity against *Staphylococcus aureus*. The present work is an attempt to justify that the method of extraction influences the efficacy of the plant extracts.

Keywords: Antimicrobial, *Elaeodendron*, ethnomedicine, Soxhlet extraction

INTRODUCTION

The use of plants as medicine has a long history. Recent generations have seen a great deal of research done on the plants that have been traditionally used or documented in ancient texts (Mishra *et al.* 2018). Plants grown for aesthetics can be found in specimen exhibits, houseplants, cut flowers, gardens, and landscape design projects. Plants grown for their aesthetic and floral qualities can also have medicinal and nutritional value. There has been a resurgence of interest in the use of therapeutic garden settings with medicinal value to enhance the healing process that occurs in healthcare settings (Sharifi-Rad *et al*. 2017). Even in the current period, many populations still rely on traditional plant-based therapy, especially in developing countries. This is largely because traditional medicines are scarce and expensive in developing countries. About 80 percent of individuals in industrialized countries utilise conventional medicine, which comprises substances derived from medicinal plants (Jennifer *et al.* 2022).*Elaeodendron* Jacq. is a genus in the Celastraceae family. The Celastraceae belongs to the order Celastrales and consists of approximately 98 genera and 1350 species mainly distributed in the tropics and temperate regions of the world (Simmons, 2004; Mabberley, 2017; Savino *et al.* 2023). In India, it is represented by 12 genera and 82 species (Ramamurthy, 2000). There have been a number of studies documented on ethno- medicinal value of the species.

The decoction stem bark has been extensive used against snake bite in the tribal pockets of Shahapur and Jawhar forest division of Thane forest circle, Maharashtra, India (Kolhe *et al*. 2022).The root bark of other species of *Elaeodendron* also been used against chest pain.Phytochemical screening of bark yielded tannins. The leaf juice has been used against snake bite in the tribal pockets of Ahmednagar District, Maharashtra (Gayake *et al.*2013). With this background, the present study has been undertaken to screen the antimicrobial potential of stem bark of the species.

MATERIALS AND METHODS

Plant collection and extract preparation

The bark of *Elaeodendron paniculatum* Wight & Arn. was collected fresh in the early hours in October 2022, from Karnatak University campus, Dharwad, Karnataka. The plant was identified with

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the aid of flora (Ramamurthy, 2000) and authenticated by Dr. Sidanand V. Kambhar, Government First Grade College, Raibag. Herbarium specimen was prepared and was deposited in the Herbarium of Department of Botany, Government First Grade College, Raibag.

The coarsely powdered plant material was weighed and packed ina filter paper and introduced into the soxhlet apparatus. Serial extractions were made using the different solvents in the order of their increasing dielectric constants (Kokate, 1993). The completion of extraction was understood by observing the siphoning side tube of the apparatus containing colourless solvents. Then the extraction was stopped and extract was made into solvent free by heating on water bath and finally kept in an oven below 50^0 C. this dried extract was weighed to know the percentage on yield and this was further diluted before using it for antimicrobial screening.

Preparation of test solution and culture medium

The completely dried extracts were weighed and dissolved in DiMethyl Formamide (DMF) and distilled water in the ratio of 1:4 to get the required concentration of 200 mg/mL, 100 mg/mL and 50 mg/mL. From these, effective concentrations were obtained by making sterile disc to imbibe 10 µL of each solution. These imbibed discs were further used for antimicrobial screening tests. All the extracts were tested against five bacterial strains, they are *Bacillus subtilis*, *Escherichia coli*, *Micrococcus flavus*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* and one fungal strain *i.e*., *Aspergillus niger.* The bacterial strains were obtained from National Chemical Laboratory and fungal strain was obtained from stock culture maintained in the Mycology laboratory, Department of Botany, Karnatak University, Dharwad. Preparation of bacterial, fungal broth and culture medium were prepared by a method suggested by Aneja (2003).

Antimicrobial activity

The antimicrobial activity was studied by disc diffusion method (Bauer *et al.* 1996) and inoculum suspensions of all the strains were prepared. For all the bacterial strains and the fungal strain, peptone water was selected as the growth medium and sterilized distilled water was taken as the growth medium for the fungus *A. niger*. Nutrient agar (Hi-media) was selected as the bacterial medium and Patato Dextrose Agar (Hi-media) as fungal medium. Twenty milliliter of the sterilized medium was poured in the pre-autoclaved petri plates and allowed to solidify. The 12 h culture broth was swabbed on the agar surface. Sterile discs impregnated with 10µL of the extract were placed on the media and gently pressed down to ensure the contact with the medium. Then the plates with bacterial strains were incubated at 30^0 C for 24 h and 48 h for fungi. The zone of inhibition was noted. Strephtomycin was used as standard for bacteria and Nystain was used as standard for fungi.

RESULTS AND DISCUSSION

All the three concentrations of acetone extract exhibited moderated activity against *Bacillus subtilis* and *Micrococcus flavus*. Higher concentration of acetone extract showed maximum activity against *Staphylococcus aureus* and low activity against *Micrococcus flavus*. Ethanolic extract showed good activity against *Staphylococcus aureus* and *Micrococcus flavus* (Fig. 1). But *Bacillus subtilis* did not show any response to 50 mg/ml concentration. There was no inhibitory effect in aqueous extract against tested bacterial strains. However none of the extracts has shown activity against *Escherichia coli* and *Pseudomonas aeruginosa*. The activity against fungi is not significant in any of the extracts. There was no extract obtained in the chloroformic extract (Table 1).

It is generally known that higher plants contain antibacterial compounds (Srinivasan *et al.* 2001). Since plant-derived medications have significantly improved human health, plants have served as an inspiration for new medicinal molecules. Phytomedicine can be utilised in conjunction with the Unani and Ayurvedic medical systems, or it can serve as the foundation for the creation of a medicament.

The extraction method's choice of solvent has a significant impact on the subsequent isolation of

Table 1: Antimicrobial activity of bark of *Elaeodendron paniculatum*

Extract	Conc· (mg/ml)	Zone of inhibition (mm)[a]					
		B. subtilis	*E-coli*	*M. flavus*	*P. aureginosa*	*S. aureus*	*A. niger*
Acetone	200	10.00±00	NI	09.00±0	NI	13.36±0.26	NI
	100	8.26±0.14	NI	08.00±0	NI	10.00±0	NI
	050	7.16±0.12	NI	7.33±0.33	NI	7.40±0.35	NI
Ethanol	200	8.36±0.18	NI	8.00±0	NI	9.00±0	NI
	100	6.83±0.16	NI	7.70±0.2	NI	8.00±0	NI
	050	NI	NI	7.33±0.33	NI	7.33±0.33	NI
Aqueous	200	NI	NI	NI	NI	NI	NI
	100	NI	NI	NI	NI	NI	NI
	050	NI	NI	NI	NI	NI	NI
Control		NI	NI	NI	NI	NI	NI
d (standards)		21±00	21.63±0.31	21.9±0.94	21.00±0	22.00±0	18±0

[a]= values are in mean ± SE of three determinants
C= 10 µL/disc; NI= No inhibition; d= Streptomycin (10 µg/disc) for bacteria and Nystain (100 units/disc) for fungus; Control= DMF + water (1:4)

botanical components from plant material. Traditional healers typically utilize water as the solvent, however in the current investigation, acetone and ethanol extracts has shown consistently good antibacterial efficacy against *Staphylococcus aureus* than those extracted with other solvents. Other species of *Elaeodendron* shows significant amount of compounds and their activity against different organisms, such as the species of *E. buchananii* stem bark ethyl acetate crude extract showed promising antibacterial activity against *Neisseria meningitides* with minimum inhibitory concentrations (MIC) value of 31.25 µg/mL. (Odak *et al.*2018).

The antibacterial activity of an acetone extracts of *E. croceum* bark against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, and *Escherichia coli*. The extract exhibited antimicrobial activity against the microorganisms examined, with maximum activity levels ranging from 8.0 µg/mL to >275.0 µg/mL. (Eloff, 2000). Simililarly, Kaikabo *et al*. (2009) analyzed the antimicrobial properties of *E. croceum* bark acetone extract against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Enterococcus faecalis*. Screening for MIC (minimum inhibitory concentrations) was carried at values ranging from 0.02 mg/mL to 2.5 mg/mL.

The antibacterial activity of leaves acetone extracts *E. croceum* against *Bacillus anthracis*. The extract demonstrated antibacterial activity with a MIC of 0.3 mg/mL and total antimicrobial property of 290.0 mg/mL. and (Elisha *et al.*2016). Stem bark and leaves aqueous extract of *E. croceum* used against *Staphylococcus aureus*, *Enterococcus faecalis*, *Shigella flexneri*, *Klebsiella pneumoniae*, *Salmonella typhimurium* and *Proteus vulgaris*. The extracts were active against all the pathogens tested, with zones of inhibition that range from 13.0 to 15.7 mm (Odeyemi and Afolayan, 2017). The antibacterial activity of *E. croceum* extracts demonstrates the species' potential as an herbal medication against bacterial infections (Jennifer *et al.* 2022).

The antimicrobial property of *E. transvaalense* stems bark extracts were assessed against various organisms (McGaw *et al*. 2000; Khumalo *et al*. 2019; Maroyi and Semenya, 2019). These findings supported the traditional usage of this species as an herbal remedy for venereal illnesses, sexually transmitted infections, stomach pains, diarrhoea, sore throat, skin infections, and wounds (Tshikalange *et al.* 2005; Steenkamp *et al.* 2007; Steenkamp *et al.* 2016). Based on these review, it has been understood that, the different species of *Elaeodendron* have significant medicinal value. Hence it has antiviral, anti-HIV, anticancer, antiproliferative, antioxidant, anti-inflammation, anti-plasmodial, cytotoxic,

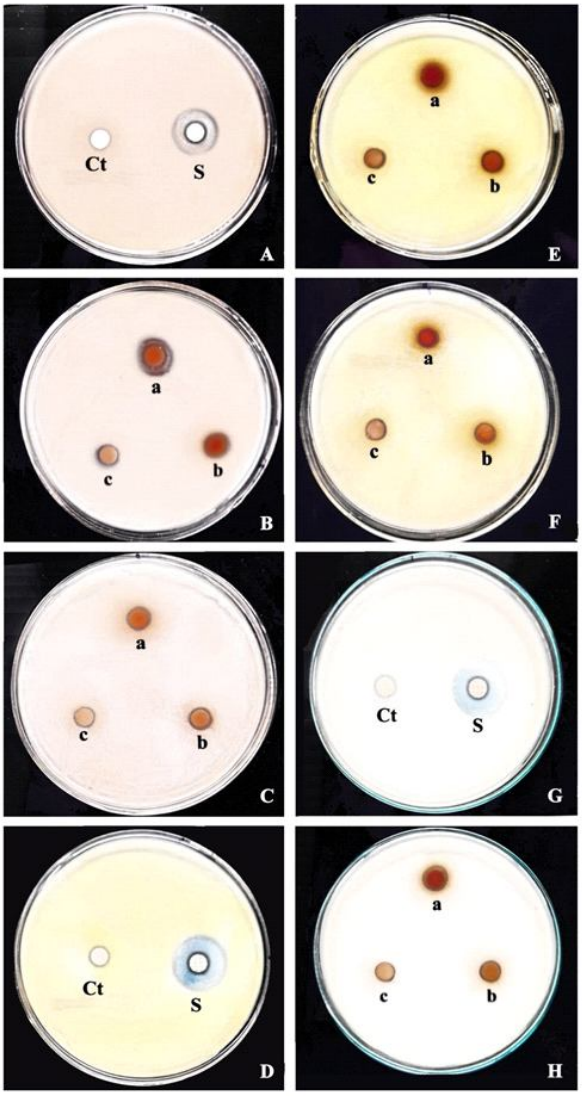

Fig.1:A. Control and standard for *Staphylococcus aureus*, **B.** effect of acetone extract on *S. aureus*, **C.** effect of ethanol extract on *S. aureus*, **D.** Control and standard for *Micrococcus falvus*, **E.** effect of acetone extract on *M. falvus*, **F.** effect of ethanol extract on *M. falvus*, **G**. Control and standard for *Bacillus subtilis*, **H.** effect of acetone extract on *B. subtilis*. **Ct**= Control, **S**= Standard, **a**= 10 µL/disc (200mg/ml), **b**= 10 µL/disc (100mg/ml), **c**= 10 µL/ disc (050mg/ml)

antifungal, anti-arthritic, and antibacterial activities in them (Jennifer *et al.* 2022). The present investigation on *Elaeodendron paniculatum* confirms the activity of this extract against microorganisms and it is further suggested to focus on the bioactivity guided separation and identification of the active components.

CONCLUSION

The results indicated that the bacterial strains were more susceptible than the fungal strains. Most of the pathogenic strains are developing resistance against antibiotics and also introduction of synthetic drugs are responsible for various side effects. Now a days, herbal drugs are being introduced to find out remedy for most of the diseases and can be used a substitute for the synthetic drugs. The present results showed that the extract of the screened plants possess some chemical components which can act against bacteria and fungi. Antimicrobial properties of these plants can be exploited further in the preparation of naturaltherapeutic agents against pathogenic bacteria.

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DECLARATIONS

Conflict of interest: Authors declare no conflict of interest.

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