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Antimicrobial Activity and Priliminary Phytochemical Studies on Blepharis repens (Vahl) Roth

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Abstract
Traditional medicine is the main source of medical care for a great percentage of the population of the developing world. Medicinal plants have been a valuable source of natural active phytoconsituents that play an important role in treatment of many human diseases. In the present study, stem (St), leaf (Lf), root (Rt), and fruit (Ft) of *Blepharis repens* (Vahl) Roth was evaluated for its phytochemicals and antibacterial potential. The Soxhlet extraction was done by using three different solvents viz., acetone, methanol and water. Qualitative phytochemical analysis of the crude powder extracts of *B. repens* stem, leaf, root and fruit was carried out for various phytoconstituents. The antimicrobial activity was evaluated by an agar disc diffusion method against four bacterial strain and two fungal strain. Qualitative phytochemical analysis shows presence of secondary metabolite in extracts. The methanol extracts showed better antimicrobial activity than acetone and aqueous extracts. This may be because of the difference in the presence of phytoconsituents present in them.
Keywords: *Blepharis repens*, antimicrobial activity, herbal medicine, phytochemical analysis.

Introduction

Medicinal plants have been useful in the development of new drugs and continue to play an valuable role in the drug discovery process (Cragg *et al*., 1999). Herbal medicines have already formed the basis of therapeutic use in developing countries, but recently have also seen an increase in the use of herbal medications in the developed world as well. This is mainly because these herbs/plants are relatively cheap, easily available and their uses are dependent on ancestral experience. India is a varietal emporium of medicinal plants and is one of the richest countries in the world as regards to genetic resources of medicinal plants. All known types of agro climatic, ecological and edaphic conditions are met within India. The biogeographic position of India is unique which makes India rich in all the three levels of biodiversity (Krishnaraju *et al*., 2006). Some studies focusing on the investigation of traditional Indian medicinal plants have resulted in the identification of new sources of therapeutic agents (Ahmad *et al*., 2001). Plants are recognized for their ability to produce a wealth of secondary metabolites and mankind has used many species for centuries to treat a variety of diseases. The curative properties of herbs lie in secondary metabolites with *in situ* functions including growth regulation, inter and intra-specific interactions and defense against predators and infections. Many of these natural products have been shown to present interesting biological and pharmacological activities and are used as chemotherapeutic agents or serve as the starting point in the development of modern medicines (Verpoorte *et al.,* 1998, 2000). Herbs are safe, less toxic, economical and a reliable key natural resource of drugs all over the world (Cragg *et al.,* 1999). Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of disease, but are also faced with adulteration and side effects. Therefore, there is a need to search new safe infection fighting strategies to control microbial infection. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and an urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Rojas *et al*., 2003). Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity. Thus, plants can be investigated for their phytoconstituents and antimicrobial efficacy.

Material and method

Several seasonal surveys will be made to collect the plant material for the experimentation.

Collection of plant materials: Collection will be carried out from forest localities from Yavatmal district. Identification will be made with the help of standard floras (Karthikeyan and Kumar, 1993, Kamble and Pradhan, 1988, Naik, 1998 and Singh *et al.*, 2001).

Preparation of powder and extract of various parts of selected plant

The collected plant material will be shade dried and mechanically powdered and stored in an airtight container.

Various extracts will be prepared according to the methodology of Sadashivan and Manickam (2005), will be subjected to our entire studies. The shade dried plants parts will be allowed to pulverization to get coarse powder. The coarse powder material will be subjected to Soxhlet extraction separately and successively with acetone, methanol and aqueous extracts. These extracts will be concentrated to dryness in flash evaporator.

Qualitative phytochemical analysis

The plants are prescribed as remedies on different health ailments. Study of bioactive component will be made to understand the medicinal value of the plant materials will be screened for bioactive compounds like, proteins, amino acids, carbohydrates, alkaloids, steroids, cardiac glycosides, anthroquinone, glycosides, saponine, flavonoids, terpenoids, quinine and coumarins. Above phytochemicals analysis will be carried out using standard procedure (Harborne, 1998, Kokate, 2005, Sadashivan and Manickam, 2005).

Antimicrobial activity

Antimicrobial activity will be carried by using antibiotic sensitivity Agar (Hi-Media) by disc diffusion method (Elizabeth, 2005) against four bacterial and two fungal strains.

Results and Discussion

Phytochemical study of *Blepharis repens* showed the presence of carbohydrate, proteins, alkaloid, flavonoids ,steroid and saponin. Alkaloid, flavonoid and steroid present in all part of plant extract. Tanin and phenolic compound present in methanol and aqueous extract except in root. Saponin present in all part of plant in aqueous extract only. For antimicrobial activity methanol, acetone and aqueous extract of all part of plant extracts was tested with four bacterial strain- *E. aerogenes, P. aerogenosa, E. Coli, S. aurius* and two fungal strain- *F. oxysporum* and *F. proliferatum*. Methanol extract shows better antimicrobial activity than acetone and aqueous extract. Methanol extract of stem shows higher zone of inhibition for *E.coli* than other microbes, methanol extract of leaf shows higher zone of inhibition for *E. aerogenes* , methanol extract of root and fruit shows higher zone of inhibition for *F. proliferatum* than other microbes. This is because of the difference in the presence of phytoconsituents present in them.

Table 1: Qulitative phytochemical analysis of *B. repens* extracts.

Sr.No.	Test		Plant parts	Acetone extract	Methanol extract	Aqueous extract
1	Carbohydrate	Benedict's Test	St	+	+	+
			Lf	-	+	+
			Rt	+	+	+
			Ft	+	+	+
2	Protein	Million's Test	St	-	-	+
			Lf	-	-	+
			Rt	-	-	+
			Ft	-	-	+
3	Antraquinone glycoside	Borntrager's Test	St	-	+	-
			Lf	-	+	-
			Rt	-	+	-
			Ft	-	+	-
4	Cardiac glycoside	Kellar-Killiani Test	St	+	+	+
			Lf	+	+	+
			Rt	+	-	+
			Ft	+	+	+
5	Coumarins		St	+	+	+
			Lf	+	+	+
			Rt	-	+	+
			Ft	+	+	+
6	Quinone		St	+	+	-
			Lf	-	+	-
			Rt	-	+	-
			Ft	+	+	-
7	Steroids	Salkowski Test	St	+	+	+
			Lf	+	+	+
			Rt	+	+	+
			Ft	+	+	+
8	Alkaloids	Hager's Test	St	+	+	+
			Lf	+	+	+
			Rt	+	+	+
			Ft	+	+	+
9	Flavonoids	Lead acetate Test	St	+	+	+
			Lf	+	+	+
			Rt	+	+	+
			Ft	+	+	+
10	Tannins and Phenolic compounds	$FeCl_3$ Solution Test	St	-	+	+
			Lf	-	+	+
			Rt	-	+	-
			Ft	-	+	+
11	Saponin	Foam Test	St	-	-	+
			Lf	-	-	+
			Rt	-	-	+
			Ft	-	-	+

Table 2: Antimicrobial activity of *B. repens* extracts.

Sr.No.	Name of microorganisms	Extracts	Diameter of zone of inhibition in mm			
			St	Lf	Rt	Ft
1	*E. aerogenes*	Aq	9	7	8	10
		Ac	12	10	11	13
		Me	12	18	11	16
2	*P. aerogenosa*	Aq	10	11	6	10
		Ac	12	15	10	12
		Me	14	15	11	12
3	*E. Coli*	Aq	11	6	10	8
		Ac	12	8	11	10
		Me	13	9	11	14
4	*S. aurius*	Aq	9	10	6	8
		Ac	10	11	6	12
		Me	13	12	10	12
5	*F. oxysporum*	Aq	10	11	6	12
		Ac	12	12	10	12
		Me	12	14	6	14
6	*F. proliferatum*	Aq	6	12	8	10
		Ac	11	11	9	10
		Me	12	13	9	11

Note : St - Stem , Lf – Leaf , Rt – Root , Ft – Fruit , Aq – Aqueous , Ac – Acetone , Me – Methanol

Fig. 2.1: Antimicrobial activity of stem extract of *B. repens*

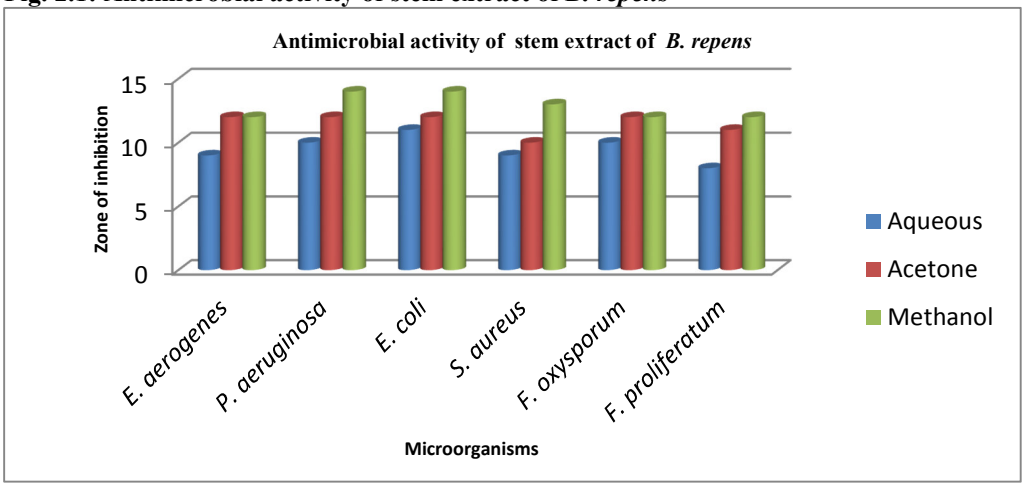


Fig. 2.2: Antimicrobial activity of leaf extract of *B. repens*

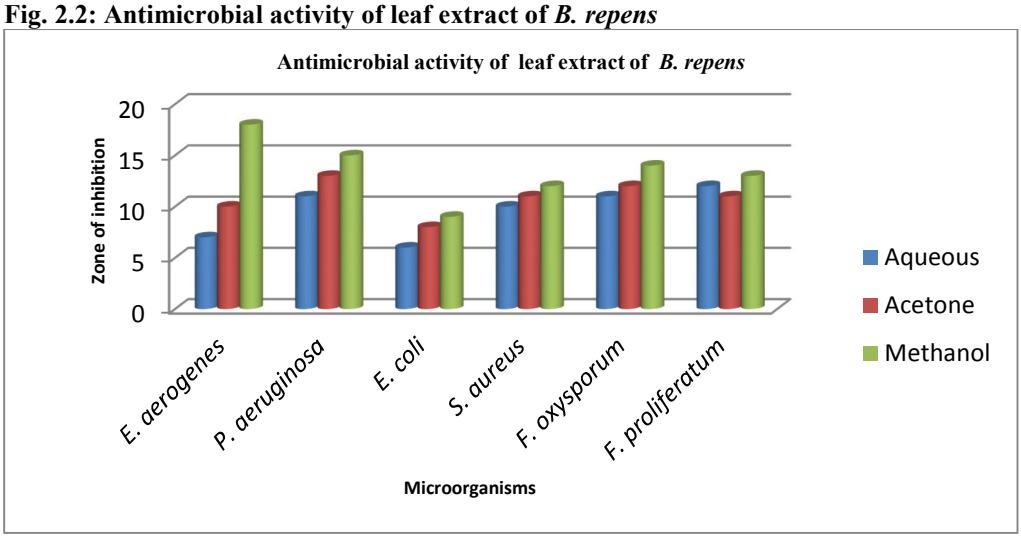


Fig. 2.3: Antimicrobial activity of root extract of *B. repens*

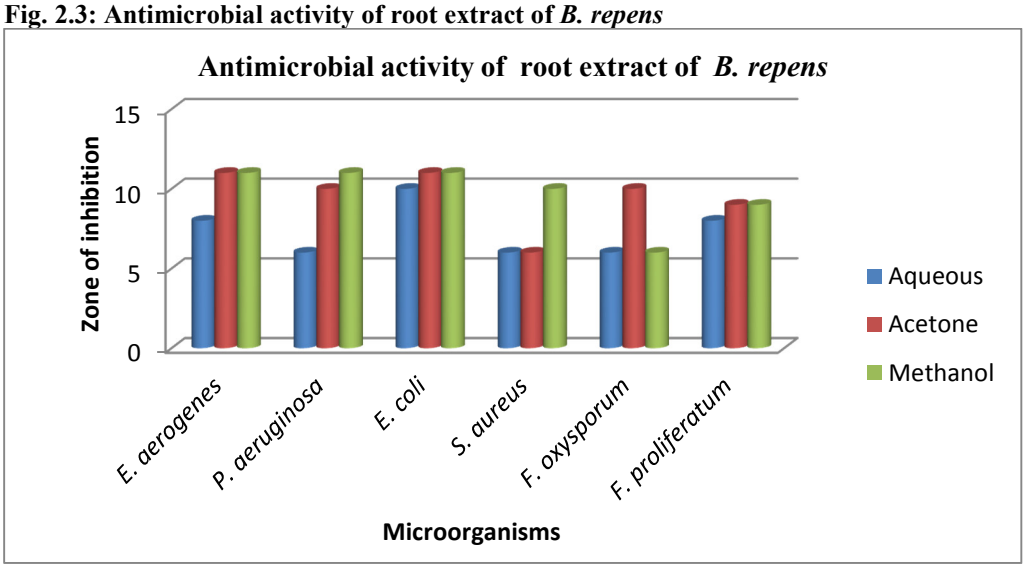
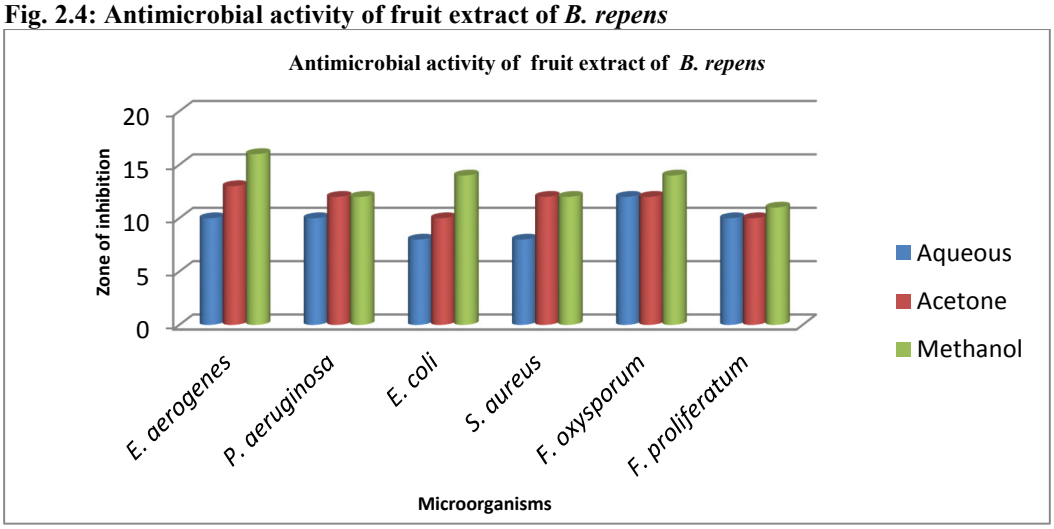


Fig. 2.4: Antimicrobial activity of fruit extract of *B. repens*



Conclusion

The results obtained from the present investigation indicate that, the plant rich in alkaloids, flavonoids, steroids, saponin. They are known to show medicinal potential and antimicrobial activity for selected microbes. Thus the plants under investigation showed their medicinal potential and can be a source of useful drugs. More phytochemical research work is required for isolation, purification and characterization of biologically active compounds. Since the plant, *Blepharis repens* is useful in traditional medicine for the treatment of various ailments; it is need of time to standardize the plant for development of quality control parameters.

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