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Conservation of Biodiversity with Reference to Indigenous Herbal Therapeutic Agents

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Abstract Biodiversity with reference to ethnic therapeutic agents is a much studied phenomenon. In the present study, commonly available herbs like *Aloe vera*, *Andropogum citratum*, creeper like *Piper betel* and tree like *Terminalia arjuna*, known to have healing effects, were checked for their antimicrobial activity. Aqueous extracts of suitable plant parts were checked for antimicrobial activities against selected Gram positive and Gram negative microbial pathogens. Maximum activity was shown by *Terminalia arjuna* against *Pseudomonas aeruginosa*. The antimicrobial activity of plant extracts was compared with that of standard antibiotics. *Piper betel* inhibited maximum number of pathogens showing equivalence to Nalidixic acid. The study emphasizes the fact that nature has cure against most natural invasions and the need for preserving and propagating such ethnic medicinal agents once again comes to fore.

Keywords: *therapeutic agent, antimicrobial activity, aqueous extract, inhibition zones, preservation and propagation, ethnic medicinal agents*

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1. Introduction

Biodiversity with reference to ethnic therapeutic agents is a much studied phenomenon. Indigenous medicinal plants in India have been established world wide as excellent antimicrobial agents. Majority of commercially important medicinal crops are cultivated in India because of its geographical location and suitable climate. Plants offer a cure to improve physical and mental health, maintain it, help in social adjustment, educational training, occupational status, recreation and in developing a leisure hobby. Plants suggest a dynamic stability through change [2]. About 70–80% of people worldwide rely chiefly on traditional, largely herbal, medicine to meet their primary healthcare needs [6,14]. The global demand for herbal medicine is growing [17]. The market for Ayurvedic medicines is estimated to be expanding at 20% annually in India [18], while in China (Yunnan) has grown by 10 times in the last 10 years [13]. This may be attributed to the increasing human population and the frequently inadequate provision of allopathic medicines in developing countries [10]. Significance of medicinal and aromatic plants has also been enhanced by higher standard of living and potential carcinogenic hazards exhibited by synthetic chemicals. Plants provide drugs at cheaper rates, are safer, relatively less toxic with least or no side effects. It is reported that jadibooty or roots of about 680 species are being exploited for medicinal purposes either by tribals or by ancient medical practitioners on a commercial or on a minor scale [4]. Traditional Maharashtrian homes use jadibooties having medicinal properties, which are dried leaves, powders or scrapings of readily available herbs and plant parts, used to treat common infections. Various active compounds (or their semi-synthetic derivatives) derived from medicinal plants have been assessed for their efficacy and tolerability in the treatment of breast cancer, antitumour activity, as antineoplastic agents used as single agents or in combinational therapies, in treatment of localized or metastatic breast cancer. [12]. Medicinal plants provide an enormous potential bioresource in modern medicine and agriculture, because of their bioactive phytochemicals, a significant number of which are actually produced by associated microbes or through interaction with their host called as plant microbiome [11].

In the present study, very commonly available herbs which are known magical jadi booties like *Aloe vera* (Korfad), *Andropogum citratum* (Gavati Chaha), creeper like *Piper betel* (Kapoori Paan) and tree like *Terminalia arjuna* (Arjun), being used for human welfare, were selected for checking their antimicrobial activity.

Aloe vera, famous for its medicinal and commercial value is available in plenty in Sangli region, as a weed. People domestically use its pulp in raw, untreated form as a conditioner for hair and skin purifier in acne. It is known to treat cough.

Andropogum citratum, is a common garden plant. Its leaves are used commonly in household preparations, especially tea, as it is known to give a strong, delicious aroma and taste, cures sore throat and cough.

Piper betel is planted on a large scale in Malgaon and Kasbe Digraj. It is of great significance in India, in pujas, traditional functions, rituals, as a mouth freshener, probably because of its great digestive value, role as a reliever of stomach-ache etc. Its leaves contain a large number of amino-acids and are eaten raw after food to stimulate salivation.

Terminalia arjuna trees are huge, commonly found in the western region of the district and are known to be useful in reducing fevers, congestions and treating wounds.

Aqueous extracts of *Aloe vera*, *Andropogum citratum*, *Piper betel* and *Terminalia arjuna* were checked as antimicrobial agents against common bacterial pathogens like Gram positive *Bacillus subtilis*, *Staphylococcus aureus*, *Micrococcus luteus* and Gram negative like *Pseudomonas aeruginosa* and *Proteus vulgaris*. The use of these plants as antibacterial agents, has been checked in this study.

2. Material and Methods

This study was carried out in the Department of Microbiology, Bharati Vidyapeeth's, Dr. Patangrao Kadam Mahavidyalaya, Sangli, Maharashtra, India.

2.1. Survey and Selection of Medicinal Plants for This Study

The plants were collected from areas in and around Sangli, Maharashtra State, India. The Sangli district comes under Deccan plateau geographic region. The area of investigation is located at the latitude of 16.8670° N and the longitude of 74.5670° E (decimal based coordinates), elevation of 545 m (1788 ft). It is situated in the river basins of the Warana, Krishna rivers and is one of the greenest areas of the country. The climate ranges from the rainiest in the Chandoli (Shirala) region, which has an average annual rainfall of over 4000 mm, to the driest in Atpadi and Jath tehsils where the average annual rainfall is about 500 mm. The vegetation cover varies from the typical monsoon forest in the western parts to scrub and poor grass in the eastern parts. Variations in the altitude and rainfall influence biodiversity.

Survey of medicinal plants was done by visiting various sites. Availability of these plants, the seasons in which they are present in ample and the purpose for which they are used, have been taken into consideration. *Aloe vera*, *Andropogum citratum*, *Piper betel* and *Terminalia arjuna* were selected for the present study;

Preparation of aqueous extract: Aqueous extracts of *Aloe vera*, *Andropogum citratum*, *Piper betel* and *Terminalia arjuna* were prepared using known weights of fresh leaves, washed with sterile distilled water, crushed in a grinder and suspended in known amount of sterile distilled water. Extracts were filtered through muslin cloth and dried.

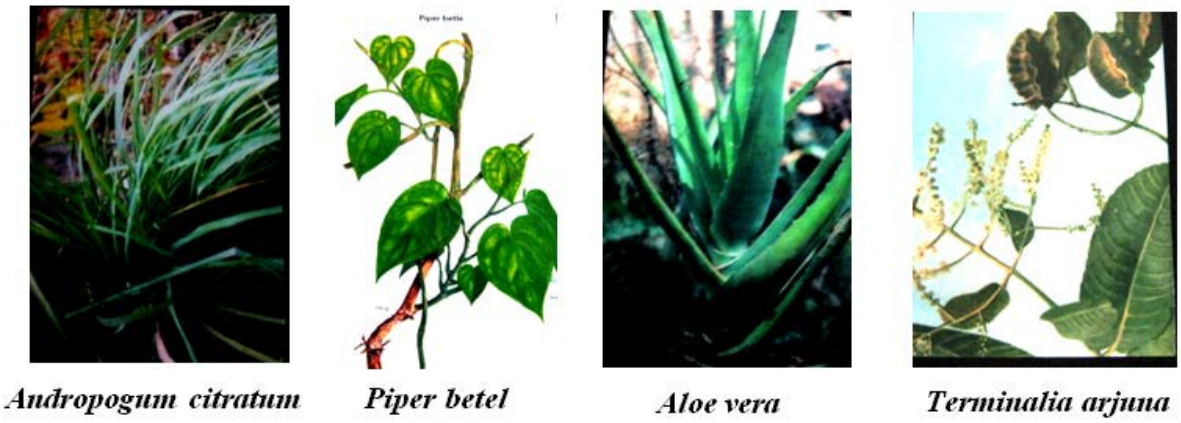

Plate I. Sample plants used for the study

Isolation of pathogens: was carried out in following steps;

- Samples were collected from the Microbiology laboratory.
- Samples were immediately used for further processing. In case of delay, they were preserved by refrigeration (at 10°C).
- Isolation of the micro-organisms was carried out using corresponding media.
- Identification of the typical isolates was carried out using different biochemical media [1,5,8,9,15].
- The identified cultures were maintained and preserved on antibiotic assay medium for further study.
- Sensitivity of these isolates to plant extracts and antibiotics was studied using diffusion assay methods.

Detection of anti-microbial activity of the plant extracts against isolates: Agar-cup diffusion assay method [7].

- Suspension of young culture of each isolate, in 0.1 ml amount, was spread separately on sterile nutrient agar plates.
- Cups were aseptically made with sterile cork borers (diameter 8 mm).
- Aqueous extract powders were diluted 1 **:** 10 with the help of sterile distilled water.
- 0.1 ml of each extract was aseptically added to corresponding cups and the plates refrigerated for 15 minutes.
- Incubation at appropriate temperatures for prescribed time was carried out.
- Plates were observed for zones of inhibition surrounding the cups.
- Diameters of the inhibition zones were recorded.

Detection of sensitivity of the isolates to antibiotics: Disc diffusion assay method [3].

- Suspension of young culture of each isolate, in 0.1 ml amount, was spread separately on sterile nutrient agar plates.
- Antibiotic discs were placed with gentle pressure, on the surface and the plates refrigerated for 15 minutes.
- Incubation at appropriate temperatures for prescribed time was carried out.
- Plates were observed for zones of inhibition surrounding the discs.
- Diameters of the zones were recorded.

Standard antibiotics: Standard antibiotic discs, commonly in use, for treating various infections were used as a control, to see the response of each of the isolates. Disc diffusion assay method was used for checking the sensitivity to standard antibiotics.

On the basis of size of zone of inhibition, organisms were concluded to be sensitive or resistant to the drug or the material under investigation [19].

3. Observation and Results

Antibacterial activity of standard antibiotics and plant extracts against bacterial pathogens by agar diffusion method

Plant Extract		*B.s*	*S.a*	*M.l.*	*S.t.*	*SpB*	*S.f.*	*P.a*	*P.v*	*S.m.*	No. of orgs. inhibited
Aloe vera		13.5 ±1.87	-	12.66 ± 1.63	-	-	-	-	-	-	2
Andropogum citratum		16.33 ±2.25	-	15 ± 4.33	20.33 ± 1.63	-	-	-	-	-	3
Piper betel		-	15.33 ±2.16	-	-	19 ± 2.28	16.66 ± 1.21	-	15.33 ± 1.86	15.33 ± 2.73	5
Terminalia arjuna		-	14.83 ±2.04	15.83 ± 1.47	-	-	-	19.16 ± 1.94	-	-	3
A' Biotic	Conc in mcg	*B.s*	*S.a*	*M.l.*	*S.t.*	*SpB*	*S.f.*	*P.a*	*P.v*	*S.m.*	
CX	30	14.33 ± 1.75 (R)	17.5 ± 1.87	19.83 ± 1.16	-	20.83 ± 1.16	22.5 ± 1.04	21.33 ± 1.63	28.16 ± 0.98	-	6
CP	5	-	-	-	32.16 ± 1.16	-	-	26.83 ± 1.16	37.66 ± 1.86	25 ± 1.41	4
CT	30	29.66 ± 1.86	24.66 ± 1.86	-	18.33 ± 1.03	-	22.16 ± 1.16	-	-	-	4
NT	30	18.83 ± 1.83	19.5 ± 1.04	23.83 ± 1.94	18.33 ± 0.81	19.83 ± 1.16	18.5 ± 1.04	-	23.66 ± 1.966	19.33 ± 1.63	8
OF	5	26.33 ± 4.22	20.16 ± 1.16	24.16 ± 1.16	19.5 ± 1.37	-	21.5 ± 1.04	-	-	20.16 ± 1.47	6
NX	10	19.83 ± 1.16	33 ± 2.366	21.5 ± 1.04	32.5 ± 2.25	40.5 ± 1.51	-	39.33 ± 1.21	-	19.5 ± 1.04	7
NA	30	18.33 ± 2.33	21.33 ± 1.211	18.53 ± 1.47	-	21.66 ± 1.21	18 ± 1.26	-	-	-	5
NF	300	17.5 ± 1.37	20 ± 1.4142	21.33 ± 1.03	-	-	18.5 ± 1.04	-	-	-	4
No. of Antibiotics to which organisms respond		6	7	6	5	4	6	3	3	4	

Key:
Values are mean ± S.E.M. (n=3). "-" refers to no antibacterial effect of medicinal plant to the mentioned bacterial strain at mentioned dose. "R" refers to resistant.
CX/Ce-Cephotaxime, CP-Ciprofloxacin, CT/Ca-Ceftazidime, NT-Netilmicin,Netillin, OF-Ofloxacin, NX-Norfloxacin, NA-Nalidixic acid, NF-Nitrofurantoin,
B.s. – Bacillus subtilis, S.a. – Staphylococcus aureus, M.l.- Micrococcus lutea, S.t.- Salmonella typhi, SpB - Salmonella paratyphi B, S.f.- Shigella flexneri, P.a. – Pseudomonas aeruginosa, P.v. – Proteus vulgaris, S.m.- Srratia marsescens

4. Discussion and Conclusion

Antimicrobial activity of the plants included in the study, elicited in terms of inhibition zones by agar diffusion method, showed that *Aloe vera* and *Andropogum citratum* extracts inhibited *Bacillus subtilis, Micrococcus luteus, Andropogum citratum* extract inhibited *Salmonella typhi,* while *Piper betel* extract was found to inhibit *Staphylococcus aureus, Salmonella paratyphi B, Shigella flexneri, Proteus vulgaris* and *Serratia marscecens, Terminalia arjuna* showed activity against *Staphylococcus aureus, Micrococcus luteus* and *Pseudomonas aeruginosa*. *Piper betel* extract was found to be the most effective, inhibiting five of the test cultures used. Maximum activity was shown by *Terminalia arjuna* against *Pseudomonas aeruginosa*. On comparing with the antibiotics, it can be concluded that *Piper betel* extract shows antimicrobial activity equivalent to Nalidixic acid. Moreover, it would be interesting to explore the use of these plants as prebiotics, which are ingredients that allow specific changes, both in the composition and / or activity in the gastrointestinal microflora that confers benefits upon host well-being and health. It is a functional food component, which is conceptually an intermediate between foods and drugs [16]. These natural products may thus prove to be promising as chemotherapeutic agents and probably as prebiotics, without many side-effects.

Various active compounds and their semi-synthetic derivatives from medicinal plants have been assessed for treatment of breast cancer, antitumour activity, as antineoplastic agents, as single agents or in combinational therapies, in treatment of localized or metastatic breast cancer.

The present study was carried out to deliberate upon whether medicinal plants can be useful as an alternative

for allopathic therapy and their potential use as antimicrobial agents and how their conservation and preservation is necessary for human welfare. Further studies need to be done to deliberate upon what measures can be taken to fulfill this.

5. Suggestions

The results of the present study lead to an information on various anti-bacterial microbial properties of *Aloe vera*, *Andropogum citratum*, *Piper betel* and *Terminalia arjuna*. It helps in creating awareness towards the biodiversity, properties and use of medicinal plants, which are easily available, can be specially cultivated, protected and preserved. They do not exhibit any harmful side effects. All standard antibiotics used in this study are known to show serious side-effects. The study emphasizes the fact that nature has cure against most natural invasions. It is a need of the time to preserve and propagate such ethnic medicinal agents and protect our biodiversity.

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