TANNINS – antimicrobial CHEMICAL COMPONENTS

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ABSTRACT — Three medicinal plants, Catharanthus roseus, Terminalia arjuna and Piper betel, frequently used as home remedies and found frequently in our surroundings, were checked for their antimicrobial properties and chemically analyzed for components. Tannins were detected in the extracts of these plant parts. Tannins are plant poly phenolic compounds which bind to proteins, amino acids, alkaloids and precipitate them. They are known antimicrobial biomolecules. The antimicrobial action of extracts of suitable plant parts was investigated against a few Gram positive and Gram negative commonly pathogenic organisms. Good antimicrobial activity was observed, which may be a result of the presence of antimicrobial components in plants like tannins. Comparatively, aqueous extract of the plants was found antimicrobially more efficient than acetone extract. These results inspire novel leads, which may help in the engineering and development of pharmacologically acceptable antimicrobial agents or their classes, in future.

Index words: antimicrobial biomolecules, antimicrobial properties, chemical analysis, engineering and development, medicinal plants, polyphenolic compounds, tannins.

I. INTRODUCTION

It is an established fact that plants have healing powers and many medicinal plants have been identified to have such efficiencies. In 1950, antibiotics started being used regularly which resulted in plants being out of use as antimicrobial agents. Since ancient times, plants are always being used as raw resources for medicinal derivatives. Antimicrobial plant extracts are of interest as these phyto chemicals may be included in the arsenal of antimicrobial drugs because of their extended life span and that of antibiotics being limited. Moreover, mass awareness of problems with the over prescription and misuse of traditional antibiotics has increased.

Since 1995, naturopathy; use of plant extracts and other alternative treatments becoming more popular. Green plants are known to have broad spectrum of synthetic activity and are sources of many useful compounds [1]. Higher plants, shrubs, certain vegetables have been originally recognized as having antiseptic activity.

Extracts of many medicinal plants have shown to be very active against pathogenic bacteria [2]. Tannins have been isolated from some medicinal plants [3,4]. Tannins are alternatively called as vegetable tannins, natural organic tannins or tannoids.

Tannins are bio molecules, astringent, bitter plant poly phenolic compounds which bind to proteins, amino acids, alkaloids and precipitate them. They are known antimicrobial biomolecules. The term "tannin" has arisen from tanna, meaning oak or fir tree. The term "tannin" means a large polyphenolic compound containing sufficient hydroxyls and other groups like carboxyls, to form strong complexes with various macromolecules.

Tannins and related compounds are widely distributed in many plant species, where they protect plants from predation, function as pesticides and aid in plant growth regulation. They play an important role in ripening of fruits and aging of wine. Tannins are either gallic acid esters (molecular weight – 500 to 3,000) or proanthocyanidins (molecular weight up to 20,000).

Tannins include flavone-derived tannins with heavily hydroxylated base, polymerized to give the high molecular weight polyphenol motif that characterizes tannins. Tannins function as protein binders if they have at least 12 hydroxyl groups and five phenyl groups. Pomegranates, nuts, berries, herbs, spices, legumes, chocolates, tea and coffee are known sources of tannins.

For centuries, preparations containing Tannins as the main physiologically active ingredient are being used for treatment of human diseases. Natural products are under scrutiny today as important anti-infective agents and the structures of tannins possessing antifungal, antiviral and antibacterial activity have been identified. Moreover, synergistic relationship between active tannins and antibiotics has been demonstrated. Tannins and related compounds present themselves as new leads which may aid in the development of pharmacologically active and appropriate antimicrobial agents or their classes [5]. Tannins and poly phenolic compounds are components of medicinal and other plants, which have magical properties, keep pathogens away, act as pro and pre biotics, keep us active, healthy and enhance our immunity. Tannins may be responsible for anti-microbial activity investigated in this paper. Selected three medicinal plants with tannins have been studied in this research paper, for their antimicrobial properties against common human pathogens; Three medicinal plants, Catharanthus roseus (Sadaphuli), Terminalia arjuna (Arjun) and Piper betel (Kapoori paan) frequently used as home remedies and found

frequently in our surroundings, were checked for their antimicrobial properties against few Gram positive and Gram negative pathogens. They were chemically analyzed for components, in this research investigation. Their analysed extracts revealed the presence of tannins. It was observed from the results that comparatively, aqueous extract of the plants was found antimicrobially more efficient than acetone extract. These results inspire novel leads, which may help in the engineering and development of pharmacologically acceptable antimicrobial agents or their classes, in future. There is on-going research on potential health benefits of individual tannins.

II. MATERIAL AND METHODS

Survey and selection of medicinal plants for this study: Three plants, known to be medicinal and regionally used as domestic remedies were selected after survey in and around Sangli, after consulting, traditional practitioners, healers and users. Availability of these plants, the seasons in which they are present in ample and the purpose for which they are used, are taken into consideration. Leaves of Catharanthus roseus, terminalia arjuna and Piper betel were used during this study, after authentication by consulting the experts.

Figure 1: Catharanthus roseus, Piper betel Terminalia arjuna

Preparation of aqueous plant extracts: Plant leaves of selected plants were collected, and weighed. After washing them with sterile distilled water, they were crushed in a mixer until a fine paste was made. If required, measured amount of sterile distilled water was added part by part. The material was filtered using first a muslin cloth and then a Whatman's filter paper (no. 40). After drying the filtrate, a fine powder was obtained which was used as sample for investigation [6]. Isolation of pathogens: Pathological samples were obtained from the Microbiology laboratory and immediately used for isolation of micro-organisms using appropriate media. Pathogenic isolates were identified using appropriate biochemical media. [7, 8, 9].

The identified cultures were preserved on antibiotic assay medium (trypticase soy agar slants) for further study. Common infection causing organisms isolated included four Gram positive and eight Gram negative bacteria, one yeast and one fungal culture.

They were identified to be Bacillus subtilis, Staphylococcus aureus, Enterococcus fecalis, Micrococcus luteus as Gram positive and Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Salmonella paratyphi B, Shigella flexneri, Pseudomonas aeruginosa, Proteus vulgaris, Serratia marsescens as Gram negative while Candida albicans and Aspergillus niger as fungal isolates. Anti-microbial activity detection against isolates by Agar-cup diffusion assay method [10]. Young culture suspension of each isolate, in 0.1 ml amount, was spread separately on nutrient agar plates (bacterial culture) or Sabouraud's agar plates (fungal).

Cups were aseptically dug in the plate with diameter 8 mm sterile cork borers. Aqueous and acetone extracts were diluted 1 : 10 in sterile distilled water. 0.1 ml of each extract was added to corresponding cups aseptically and the plates kept at low temperatures 20 minutes. After incubation at 37 0 C for 24 hrs., plates were observed for inhibitory zones surrounding the cups. Diameters of these zones were recorded. Qualitative detection of Tannins in the extracts [11]: Ferric chloride test: A few drops of 10% FeCl3 solution were added to the test solution (1 ml.). Development of a dark brownish black color indicated the presence of tannins.

III. OBSERVATIONS AND RESULTS

Results were observed in the form of inhibitory zones surrounding the cups with extracts. Diameters of the inhibitory zones were noted. Numbers of isolates inhibited indicated the comparative efficiencies of aqueous and acetone extracts (Tables 1 and 2).

Table 1: Effect of extracts of *Catharanthus roseus, Piper betel* and *Terminalia arjuna* on isolates, in terms of Zone of inhibition in mm (Mean ± S.D.)

	Catharanthus roseus		*Piper betel*		*Terminalia arjuna*	
Name of the isolate	**Aqueous extract**	**Acetone extract**	**Aqueous extract**	**Acetone extract**	**Aqueous extract**	**Acetone extract**
B.subtilis	-	-	-	11.83 ± 0.98	-	11.66 ± 1.03
S. aureus	-	-	15.33 ± 2.16	-	14.83 ± 2.04	14.5 ± 1.04
Ent. fecalis	14.83 ± 1.47	-	-	-	-	16.83 ± 1.16
M. luteus	-	-	-	-	15.83 ± 1.47	19.33 ± 1.03***
E. coli	19.16 ± 1.94	-	-	-	-	20 ± 1.26
K. pneumoniae	21.66 ± 2..33	-		-	-	21.33 ± 1.03
Sal. typhi	-	-	-	-	-	14.83 ± 0.75
Sal. paratyphi B	-	-	19 ± 2.28	11.16 ± 1.16***	-	16.66 ± 1.21
Sh. flexneri	-	-	16.66 ± 1.21	12.5 ± 1.04***		13 ± 1.41
Ps. aeruginosa	-	-	-	-		-
P. vulgaris	-	-	15.33 ± 1.86	15.16 ± 0.75		-
Ser. marsescens	-	-	15.33 ± 2.73	-		-
C. albicans	14.83 ± 1.16	-	10.33 ± 1.03	-		-
Asp.niger	-	-	-	-	-	-
Total no. of orgs. Responding	4	0	6	4	3	9

Key : Values are mean ± S.E.M. (n=3), significance Vs control; * P < 0.05, significant, ** P < 0.01 highly significant, *** P < 0.001, very highly significant, "-" refers to no antibacterial effect of medicinal plant to the mentioned bacterial strain at mentioned dose.

Table 2: Comparative efficiencies of aqueous and acetone extracts of plants in inhibiting pathogenic isolates

Name of the plant extract under investigation	**Aqueous extract % efficiency**	**Acetone extract % efficiency**
Catharanthus roseus	29	0
Piper betel	43	29
Terminalia arjuna	21	64

IV. DISCUSSION AND CONCLUSION

The present research showed that extract of Terminalia arjuna prepared in acetone was the most effective of the three inhibiting nine isolates out of fourteen and the Catharanthus roseus extract was the least effective, inhibiting none.

Catharanthus roseus is found commonly in the surroundings, considered a harmless shrub with colourful flowers. Today, it has gained importance as containing anti-cancer agents like Vinchristine, Vinblastine, being used as a chemotherapeutic treating agent for various malignancies. It's aqueous extract showed inhibitory effect towards the growth of Gram positive strain of Ent. fecalis, Gram negative E. coli, Kl. Pneumonia and yeast C. albicans.

The acetone extract was not effective in inhibiting any of the test isolates. Aqueous extract of C. roseus showed 29 % efficiency and acetone extract 0 %

efficiency (Tables 1 and 2). Terminalia arjuna, known to strengthen heart muscles, is anti-microbial to an extent, may be due to factors like alkaloids, sterols and tannins.

Aqueous extract was inhibitory for the growth of Gram positive S. aureus, M. luteus and Gram negative Ps. Aeruginosa; acetone extract inhibitory for the growth of Gram positive S. aureus, M. luteus, B. subtilis, Ent. fecalis, Gram negative, E. coli, Kl. pneumonia, Sal. typhi, Sal. paratyphi B and Sh. Flexneri. Aqueous extract of T. arjuna showed 21% inhibitory efficiency while acetone extract 64% (Table 1 and 2). Piper betel, a very popular plant, leaves of which are consumed by millions of Indians, after food, is a digestive. It has recently found a very significant place in medicine. Aqueous extract was found inhibitory towards Gram positive S. aureus, Gram negative Sal. paratyphi B, Sh. flexneri, P. vulgaris, Ser. marsescens and C. albicans. Acetone extract was found inhibitory towards Gram positive B. subtilis, Gram negative Sal. paratyphi B, Sh. flexneri, P. vulgaris while C. albicans, Asp. niger were found to be resistant. Aqueous extract of P. betel showed 43% inhibitory efficiency while acetone extract 29% (Tables 1 and 2).

Comparing, aqueous extract was found to be more effective than acetone extract. Many references support the study conducted. The efficiency few plant extracts as natural antimicrobial agents and the possibility of utilizing them in medicines for treating infectious diseases caused by the test pathogens was supported by the study showing the effect of ethanol, methanol, acetone and water extracts of leaves of 11 medicinal plant species against six antibiotic resistant clinical pathogens, showing antimicrobial activity for most of the extracts.

Ethanol leaf extract showed broad-spectrum antimicrobial activity followed by that of methanol [12]. Tannins in Euphobia hirta were found to be antimicrobial against Salmonella typhi in vitro [13]. Tannins, a group of polymeric phenolic substances, can be toxic to bacteria, filamentous fungi and yeast [14].

Acetone and aqueous extracts of the stem bark of Sclerocarya birrea showed antimicrobial activity towards many tested bacterial and fungal species. The component polyphenolic compounds showed 27.2 mg/g tannic acid which is equivalent to phenols [15]. When Chitosan and Tannic acid were applied on cotton fibrous materials, individually as well as in combination, for observing their antimicrobial activity, it was found higher for Tannic acid than Chitosan [16].

Plants produce various types of secondary metabolites, many of which are antimicrobial in nature. Some of these exist in healthy plants in biologically active forms. Some occur as inactive precursors, activated only in response to tissue damage or pathogen attack Condensed tannin from Rhizophora apiculata barks subjected to antimicrobial screening test against 18 bacteria species, 12 filamentous fungal species and 4 yeasts, exhibited good inhibitory activity against certain bacterial and yeasts tested.

This suggested its use as antimicrobial natural products which may serve in developing new pharmaceuticals in food and as an antimicrobial agent against certain food spoilage organisms [17]. Lot of research is going on to check plants anew with view towards their antimicrobial usefulness. Various phyto chemicals like tannins, with inhibitory effects on microorganisms in vitro, have been found.

Alternative mechanisms of treatment and prevention of infections are necessary in today's global scenario. High resistance of pathogens towards antibiotics, calls for a new era of plant-derived chemotherapeutics against infectious agents. The results obtained in the present research inspire novel leads, which may help in the engineering and development of pharmacologically active and applicable antimicrobial agents or their classes, in future.

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