Antimicrobial and phytochemical analysis of bark of some medicinal plants

[*1] SM Shende, [2] SG Kalane, [*3] VB Bhise, [4] AR Gupta

[1] Department of Botany, Late Pundalikrao Gawali Arts and Science Mahavidyalaya, Shirppur (Jain), Dist. Washim, Maharashtra, India

[2] Department of Chemistry, Late Pundalikrao Gawali Arts and Science Mahavidyalaya, Shirppur (Jain), Dist. Washim, Maharashtra, India

[3] Department of Physics, Late Pundalikrao Gawali Arts and Science Mahavidyalaya, Shirppur (Jain), Dist. Washim, Maharashtra, India

[4] Department of Mathematics, Late Pundalikrao Gawali Arts and Science Mahavidyalaya, Shirppur (Jain), Dist. Washim, Maharashtra, India

Abstract

The plant based drugs and chemical for curing various ailment used since old human civilization. Plants and plant based medicament are the basis of many of the modern pharmaceutical industries that we used today for our various ailments. The aim of the study was to find out the bioactive chemical constituents to study the antimicrobial activity of 95% methanol extract of bark of seven medicinal plants belongs to the different families. A qualitative phytochemical analysis was performed for the detection of alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins and reducing sugar Preliminary evaluation of bark extract showed a broad spectrum of antimicrobial activity since the extract inhibit the growth of both gram positive and gram negative bacteria. The highest yield of methanolic extract was found in *Azadiracta indica* (34.39%). *Dalbergia sisoo* contained all the constituents except alkaloid. *Polyalthia longifolia* lacked alkaloid and terpenoid while *Bauhinia variegata lacked* steroid, tannin and reducing sugar. The extract of the crude extract of *Aegle marmelos* showed maximum inhibition for the species *S. aureus and* the crude extract of *Bauhinia variegata* showed maximum inhibition against *Streptococcus pneumonia* respectively. The crude extract of *Dalbergia sisoo* and *Bauhinia variegata* showed maximum inhibition against gram- negative bacteria *E. coli* and *Klebsiella pneumonia* respectively. Similarly, the extract of *Azadiracta indica and Dalbergia sisoo* were active against *Staphylococcus aureus* and *Streptococcus pneumonia* and the zone of inhibition of *Polyalthia longifolia* and *Bauhinia variegata* were active against *E.coli* and *Klebsiella pneumonia* respectively. This study therefore support the traditional uses of the plant in the treatment of infectious diseases.

Keywords: Bark extract, phytochemical screening, zone of inhibition

1. Introduction

Plants have been used to cure illness since before recorded history. The period between 3500 B. C. and 800 B.C. give many references of medicinal plants. The herbal medicine is "*Virikshayurveda"* compiled even before beginning of Christian era. "*Rig Veda"* one of oldest available literature written around 2000 B.C. mention the use of Cinnamon (*Cinnamomum verum*), Ginger (*Zinger officnale*), Sandalwood (*Santalum album*) etc. not only in religious ceremonies but also in medical preparation [1]. Plants and plant-based medicament are the basis of many of the modern pharmaceutical we use today for various ailments [2] the discovery of medicinal plants based on two factors. One has been development of increasing strict criteria of proof that a medicine really does what it claimed to do and other has been the identification by chemical analysis of active compound in the plant [3].

According to world health organization (WHO), more than 80% of world's population trust to depend on traditional medicines for their primary health care needs. The medicinal value of plant have chemical substances that produce certain physiological action on the human body. Medicinal and aromatic plants are the most exclusive source of life saving drugs for the majority of the world's population. To uphold the proper use of phytomedicines and to find out their potential use as a source of new drugs, it is essential to study medicinal plants, which have traditional reputation. The plant contains several medicinally important phytochemicals and hence traditionally plant parts are used in treatment of different diseases. In unani and ayurveda systems of medicine, it is used as anti-inflammatory, anti- nociceptive, anti-plasmodial, anti-lipidoxidative, anti-hyperglycemic, anti-ulcer, anti-hyper ammonic, CNS depressant [4]. The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in discovery of new anti- infective agent from higher plants [5]. It is estimated that in modern age 80% of the medical care is provided by homecare and traditional native systems of medicine and major part of these therapies involve the use of plant extracts or their active principles. Knowledge of chemical constituent of plant would be further valuable in discovering the actual value of folkloric remedies [6]. The bark is collected from the plants that grow along road side for medicinal value. The present study is an attempt to evaluate the antibacterial activity of bark against gram-positive and gram-negative bacteria.

2. Objectives

The objectives of the study was to find out phytochemical active constituents and antimicrobial activities of the following plants.

Table 1: Plants selected for study

S. No.	Scientific name	Vernacular name	Family
1	*Azadiracta indica*	Neem	Meliaceae
2	*Aegle marmelos*	Bel	Rutaceae
3	*Bauhinia variegata*	Kachnar	Caesalpiniaceae
4	*Dalbergia sisoo*	Sisham	Fabaceae
5	*Polyalthia longifolia*	Ashok	Annonaceae
6	*Rauwolfia serpentina*	Sarpagandha	Apocyanaceae
7	*Zizypus jujuba*	Bor	Rhamnaceae

3. Material and methods

On the basis of available literature, the bark is obtained from above mentioned different medicinal plants from different localities. Then collected bark is dried in the sunlight for four days to dry properly. The barks were washed thoroughly 2-3 times with running water and once with sterile distilled water. The powdered crude drug was macerated with 95% methanol. However, hexane was also used as solvent for extraction of *Azadiracta indica* [3]. These extracts were used to conduct the phytochemical evaluation, by dissolving a small quantity in the respective solvents. The phytochemical screening of the plant extracts was carried out to detect the presence or absence of certain bioactive compounds. The crude products obtained were subjected to qualitative chemical evaluation of alkaloid, glycoside, terpenoid, steroid, tannins, flavonoids, and reducing sugar.

3.1 Identification Test: The tests were done to find out the presence of different active chemical constituent such as alkaloid, glycosides, terpenoid and steroids, flavonoids, reducing sugar and tannins by the following procedure:

3.2 Alkaloid: Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloid solution produces white yellowish precipitate when a few drops of Mayer's reagent are added [7]. Most alkaloid are precipitated from neutral or slightly acidic solution by Mayer's reagent [8]. The alcoholic extract was evaporated to dryness and the residue was heated on a boiling water bath with 2 % hydrochloric acid. After cooling, the mixture was filtered and treated with a few drop of Mayer's reagent. The samples were then observed for the present of turbidity or yellow precipitation.

3.3 Glycoside: Glycoside are compound which upon hydrolysis give rise to one or more sugars (glycones) and a compound which is not a sugar (aglycone or genine). To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added, and observed for a reddish brown coloration for the junction of two layers and the bluish in green color in the upper layer [7].

3.4 Tepenoid and steroid: Four miligrams of extract was treated with 0.5 ml of acetic anhydride and 0.5ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet color was observed for terpenoid and green bluish color for steroids [7].

3.5 Flavonoid: Four miligrams of extract solution was treated with 1.5ml of 50 % Methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5 to 6 drop of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange color for flavones [7].

3.6 Tannins: To 0.5ml of extract solution 1ml or water and 1-2 drops of ferric chloride solution was added. Blue color was observed for Gallic Tannins and green Black or catecholic tannin [9].

3.7 Reducing sugar: To 0.5 ml of extract solution, 1ml of water and 5-8 drops of Fehling's solution was added at hot and observed for brick red precipitate.

All the plants extract were subjected to individual microbiological tests to ascertain their antimicrobial activity against four species of microorganism: *Escherichia coli* and *Klebsiella pneumonia* are gram-negative bacteria and *Staphylococcus aureus* and *Streptococcus pneumonia* are gram- positive bacteria. The antimicrobial activity of the extract was determine by measuring the diameter of zone of inhibition (ZI) exhibited by the extract.

The tests was carried out by cup plate method and diameter of the borer used was 6 millimeter (mm). The microorganisms used were the isolates of local hospital.

4. Result and discussion

The percentage of yield of extract and phytochemical constituent of the plants are shown in Table and 3 respectively. The highest yield of methanolic extract was found in *Azadiracta indica* (34.39) and the lowest in *Bauhinia variegata* (15.6). From the phytochemical constituent screening, the alkaloids were found *Azadiracta indica, Aegle marmelos, Bauhinia variegata, Rauwolfia serpentina, Zyzipus jujuba,* and glycosides in *Azadiracta indica, Aegle marmelos, Bauhinia variegata, Dalbergia sisoo, Polyalthia longifolia, Rauwolfia serpentina, Zyzipus jujuba.* Steroids were found in *Azadiracta indica, Aegle marmelos, Rauwolfia serpentina.* Trepenoids were observed in *Zizypus jujuba, Dalbergia sisoo*. Flavonoids were found in *Aegle marmelos, Bauhinia variegata, Dalbergia sisoo, Polyalthia longifolia*, and *Zyzipus jujuba*. Tannins found in *Dalbergia sisoo, Polyalthia longifolia* and *Zyzipus jujuba*. Reducing sugar *Dalbergia sisoo, Polyalthia longifolia*.

One percent extracts of all the plants showed some degree of antimicrobial activity. It was strongly significant against gram- positive and gram- negative bacteria. In the present study, the antimicrobial activity of barks extracts against bacterial strains were assessed by the presence or absence of inhibition zones. The crude extract of *Aegle marmelos* showed maximum inhibition for the species *S.aureus* (ZI=17

mm), then which is almost equal to antibiotic tetracyclin inhibition (19mm). The crude extract of *Bauhinia variegata* showed maximum inhibition against *Streptococcus pneumonia* (ZI=**1**4mm) which is equal to tetracyclin inhibition (ZI=15mm). The crude extract of Dalbergia sisoo and *Bauhinia variegata* showed maximum inhibition against gram- negative bacteria E.coli (ZI=13mm) and *Klebsiella pneumonia* (ZI=14mm) respectively. Similarly, the extract of *Azadiracta indica and Dalbergia sisoo* were active against *S.aureus* (ZI =13mm) *Streptococcus pneumonia* (ZI=13mm**)** and the zine of inhibition of Polyalthia *longifolia* and *Bauhinia variegata* were active against E.coli (ZI= 12mm) and *Klebsiella pneumonia* (13mm) respectively. Zone of inhibition of the individual extract are shown in the table 4. Earerly observation done by Shrinivasan *et al*. also showed the antifungal and antibacterial activity of *Azadiracta indica* [4].

Table 2: Percentage yield of extracts

Plant	Weight of raw material (gm)	Weight of the extract (gm)	Percentage of yield
Azadiracta indica(Methnolic extract)	130	37.8	34.39
Azadiracta indica(Hexane extract)	160	3.7	2.31
Aegle marmelos	80	20.3	25.37
Bauhinia variegata	100	15.6	15.6
Dalbergia sisoo	70	17.8	25.42
Polyalthia longifolia	50	15.2	30.4
Rauwolfia serpentina	74	22.6	30.54
Zizypus jujube	85	14.8	17.41

Table 3: Phytochemical constituent of plant extract

Plant extract	Glycosides	Alkaloids	Flavonoids	Terpenoids and Steroids	Tannins	Reducing Sugar
Azadiracta indica	+	+	-	+ (Sterois)	-	-
Aegle marmelos	+	+	+	+ (Steroid)	-	-
Bauhinia variegata	+	+	+	- (Terpenoid)	-	-
Dalbergia sisoo	+	-	+	+ (Terpenoid)	+ (Catecholic)	+
Polyalthia longifolia	+	-	+ Catecholic)	-	+ (Gallic)	+
Rauwolfia serpentina	+	+	-	+ (Steroid)	-	-
Zizypus jujuba	+	+	+ (Flavonoid)	+ (Terpenoid)	+ (Catecholic)	-

Where + Means Present and - Means Absent

Table 4: Antibacterial activity of individual plant bark extracts**.**

Bacterial strain	Bacteria	Plants	Zone of inhibition (mm)
Gram -Positive	*Staphylococcus aureus*	*Azadiracta indica*	13
		Aegle marmelos	17
		Bauhinia variegata	9
		Dalbergia sisoo	12
		Polyalthia longifolia	12
		Rauwolfia serpentina	10
		Zizypus jujube	6
		Tetracycline 0.5%	19
	Streptococcus pneumonia	*Azadiracta indica*	11
		Aegle marmelos	12
		Bauhinia variegata	14
		Dalbergia sisoo	13
		Polyalthia longifolia	11
		Rauwolfia serpentina	11
		Zizypus jujube	7
		Tetracycline 0.5%	15
Gram- Negative	*Escherichia coli*	*Azadiracta indica*	9
		Aegle marmelos	8
		Bauhinia variegata	13
		Dalbergia sisoo	10
		Polyalthia longifolia	14
		Rauwolfia serpentina	6
		Zizypus jujube	9
		Tetracycline 0.5%	16
	Klebsiella pneumonia	*Azadiracta indica*	10
		Aegle marmelos	7

		Bauhinia variegate	14
		Dalbergia sisoo	7
		Polyalthia longifolia	12
		Rauwolfia serpentine	10
		Zizypus jujube	8
		Tetracycline 0.5%	16

5. Conclusion

The methanolic extract of the studied plant contained many bioactive chemical constituents including alkaloids glycosids terpenoids, flavonoids, steroids and tannins.

The antimicrobial activities of medicinal plants are due to presence of flavonoids, tannins and alkaloids and these constitutes act as secondary metabolites. The extract of the crude extract of *Aegle marmelos* showed maximum inhibition for the species *S. aureus and* the crude extract of *Bauhinia variegata* showed maximum inhibition against *Streptococcus pneumonia* respectively the crude extract of *Dalbergia sisoo* and *Bauhinia variegata* showed maximum inhibition against gram- negative bacteria E.coli and *Klebsiella pneumonia* respectively. Similarly, the extract of *Azadiracta indica and Dalbergia sisoo* were active against *S. aureus Streptococcus pneumonia* and the zone of inhibition of *Polyalthia longifolia* and *Bauhinia variegata* were active against *E. coli* and *Klebsiella pneumonia* respectively.

6. References

1. Bentley R, Trimen H. Medicinal Plants, J. & A. Churchill, London. 1980, I-IV.
2. Abraham Z. Glimpeses of Indian Ethno botany, Oxford & Publishing Co., New Delhi, 1981, 308-320.
3. Holiman A. Plants in medicine. Chelsea Physic Garden. The Chelsea Physic Garden Co Ltd. 1989.
4. Shrinivasan D, Nathan S, Suresh J, Laxmanperumalsamy P. Antibacterial activtity of *Azadiracta indica* and Tamarindus indica Leaves. Asian journal of Microbiology, biotechnology and Environmental Sciences, 2001; 3(1-2):67-7.
5. Duraipandiyan V, Ayyanar M, Ignacimuthu S. Antimicrobial activity of some Ethanomedical Plants used by Paliyar Tribe From Tamilnadu, India. BMC Complementary and alternative medicine. 635.
6. Mojab F, Kamalinejad M, Ghaderi N, Vahidipour H. Phytochemical Screening of some Iranian Plants. Iranian Jouranal of Pharmaceutical Research. 2003, 77-82.
7. Siddiqui AA, Ali M. Practical Pharmaceutical Chemistry. Ist ed., CBS Publishers and Distributors, New Delhi, 1997, 126-131.
8. Evans WC. Trease and Evan's Pharmacognosy. 5th ed., Haarcourt Brace and Company, 2002, 336.
9. Iyengar MA. Study of Crude Drugs. 8th ed., Manipal Power Press, Manipal, India, 1995, 2.