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Original Research Article

DETERMINATION OF ANTIBACTERIAL ACTIVITIES AND MODE OF ACTION OF DIFFERENT FRACTIONS OF *ACACIA ARABICA LAM*

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Abstract: Micro organisms are subject to either favorable or antagonistic reactions owing to the presence of other living organisms. To avoid the side effects of synthetic drug in the recent years herbal drugs and their formulations are largely in use. Here we also used the fractions of extract of *Acacia Arabica lam* for the antimicrobial study by using the antibiotic as a standard. So; it was proposed to attempt antimicrobial study and Minimum Inhibitory concentration of fraction of bark of *acacia Arabica lam*. It is evident from literature survey that no antimicrobial method was mention on the various fractions of *Acacia arabica* bark as well as its mode of action.

In present study, an attempt has been made to derive the antimicrobial action and its mode of action by using different strains of bacteria on the different fractions of *Acacia Arabica*

The development of antimicrobial drugs represents one of the most important advances in therapeutics, both in the control or cure of serious infections and in the prevention and treatment of infectious complications of other therapeutic modalities such as cancer chemotherapy and surgery. Antimicrobial agents are frequently used before the pathogen responsible for a particular illness or the susceptibility to a particular antimicrobial agent is known. This use of antimicrobial agents is called empiric therapy and is based on experience with a particular clinical entity. The usual justification for empiric therapy is the hope that early intervention will improve the outcome; in the best cases, this has been established by placebo-controlled, double-blind prospective clinical trials.

Key words: *Acacia arabica Lam Bacillus subtilis,* Staphylococcus*aureu,* Antibacterial activity

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Introduction: The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and the Near east, but it is doubtless an art as old as mankind. Neanderthals living 60,000 years ago in present day Iraq used plants such as holly back, these

plants are still widely used in ethno medicine around the world

All plants containing active compounds are important. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, which are synthesized and deposited in specific parts or in all parts of the plant.[1, 2]

The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller. Thus, any phytochemical investigation of a given plant will reveal only a very narrow spectrum of its constituents. Historically pharmacological screening of compounds of natural or synthetic origin has been the source of innumerable therapeutic agents. Random screening as tool in discovering new biologically active molecules has been most productive in the area of antibiotics [3, 4]. Even now, contrary to common belief, drugs from higher plants continue to occupy an important niche in modern medicine. On a global basis, at least 130 drugs, all single chemical entities extracted from higher plants, or modified further synthetically, are currently in use, though some of them are now being made synthetically for economic reasons [5]

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [6]. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been adequately evaluated [8]

Plant based antimicrobials represent a vast untapped. Source for medicines and further exploration of plant antimicrobials needs to occur. Antimicrobials of plant origin have enormous therapeutic potential.[7]

Importance of Traditional Medicinal Plants:

India is the third most biologically diverse country on the earth, with 80% of more than 18 500 vascular plant species found nowhere else. Medicinal plants also called “muthi plants” forms the basis of traditional herbal medicine. India has a vast array of medicinal plants used in the treatment

of various diseases on a regular basis. There are believed to be about 27 million consumers of medicinal plants in South Africa.

Although India contains about 10% of the worlds plant diversity, relatively little work has been done on the medicinal plants from this region. There is limited research and investigation concerning the therapeutic potential of medicinal plants. Scientific investigation has led to the development of various plant-based medicines, with a worldwide contribution to health care. Information concerning the identification, uses and preparatory methods of medicinal plants has a long history of being documented mostly in the form of ethno botanical surveys. [9]

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local

used, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries.[10]

The utilization and identification of medicinal plants as therapeutic agents have various facets, aimed at improved health care. Medicinal and poisonous plants have always played an important role in the African society, for containing substances with healing properties. Active antimicrobial agents such as polygoidal, anethole, safrole and cryptoline form part of the secondary metabolites that serve as sources of defense agents against Microorganisms. Many modern medicines have their origins in plants, which have been used for millennia in treating various ailments and standing the test of time as sources of potent and powerful drugs.

These medicinal plants form an important foundation in various ethno botanical studies and phytochemical analysis in the different traditions worldwide. Medicinal plants and their derivatives contribute to more than fifty percent of all drugs used worldwide.

2.1 Collection and identification of bark

Acacia Arabica bark was collected from the village Badwai, near Karond, Bhopal M.P. and identified by Department of Botany, Safia College of Science Bhopal.Herbarium has been deposited in Department of Botany and accession number of the herb is BOT/H/2698.

Collection and identification of microorganism: Bacterial cultures of Bacillus *subtilis* (*MTCC-342*) and Staphylococcus *aureus* (*MTCC-433*) obtained from the Department of biotechnology Madhya Pradesh council of science and technology Bhopal, M.P.India, and The bacteria were maintained on nutrient broth (NB) at 37°C

Phytochemical Screening

- Hydro alcoholic Extract
- Fractions of extract
- Phytochemical screening

Experimental Work

- Selection of Bacteria
- Solvent Extraction
- Materials & Method
- Minimum inhibitory concentration
- Minimum Bactericidal concentration
- Zone of inhibition
- Dilutions for Sample
- For standard
- Loss of 260 nm absorbing material
- Membrane integrit

Results and Discussion

Phytochemical Screening

Table no .1 Qualitative chemical Test for the different extracts of *Acacia arabica* Bark

S.N.	Test	Hydro alcoholic	N.butenal	Methanol	Ethyl acetate
1. Test for carbohydrates					
Molisch's test s		+	+	+	+
Fehling test		+	+	+	+
2. Test for Glycoside					
Modified Borntrager		-	-	-	-
Kellar-killiani test		-	-	-	-
Legal's test		-	-	-	-
3. Test for Saponins					
Foam test		+	+	+	+
4. Test for alkaloids					
Mayer's test		-	-	-	-
Hager's reagent		-	-	-	-

Dragondroff's test	-	-	-	-
5. Test for Flavonoids				
Shinoda test	-	-	-	-
Alkaline reagent test	-	-	-	-
6. Test for Phenolic compounds and Tannins				
Lead acetate solution	+	+	+	+
Ferric chloride solution test	+	+	+	+
7. Test for Phytosterols and triterpenoids				
Libermann's test	-	-	-	-
Salkowski's test	-	-	-	-
LibermannBurchard's test	-	-	-	-
8. Test for Fixed oil and fats				
Saponification test	-	-	-	-

After the extraction of the plant the yield and nature of extracts was found to be:

Table 2. Yield and Nature of Extract.

Properties	Hydro alcoholic	N.butenol	methanol	Ethyl acetate
Yield	40.62%	22.62%	5.35%	2.1%
Nature	Solid	Solid	Solid	Solid
Colour	Reddish brown	Reddish brown	Reddish brown	Reddish brown

The yield was shown by hydro alcoholic extracts of the drug and different fractions of *Acacia Arabica*. The nature of the extracts in case of different fraction of *Acacia Arabica* was dry powder and reddish Brown in colour.

Results and Discussion: Crude extracts of *Acacia Arabica* was obtained and their fraction were prepared. The yield of the hydro alcoholic extract was found to be 44.01%.The fraction prepared (Ethyl acetate, Methanol, n-Butenol) from hydro alcoholic extract were then compared for their antimicrobial activity

Evaluation of the antimicrobial potential.

Two clinical isolates of *Acacia Arabica* was chosen from their largest microbial profiles and their antibacterial activity was assayed *in vitro* by Minimum inhibitory concentration (MIC) by using serial dilution method (Table 8.1, 8.2), Minimum Bacterial Concentration (MBC) (Table 8.3) by using serial dilution and Zone of inhibition by using disk diffusion method. (Table No 8.4)

Minimum inhibitory concentration (MIC)

The higher sensitivity of Gram-positive and anaerobic bacteria was confirmed by the agar dilution method. The MIC of hydroalcohalic extract, n-butenol fraction (w/v), Methanolicfraction and Ethyl acetate fraction against *Staphylococcus aureus* was found to be 5mg/ml,0.1mg/ml, 1mg/ml and 5mg/ml respectively.

The MIC of hydroalcohalic extract, N-butenol fraction (w/v), Methanolic fraction and Ethyl acetate fraction against Bacillus Subtilis was found to be 5mg/ml.

The DMSO control showed no toxic effect at 1% (v/v).

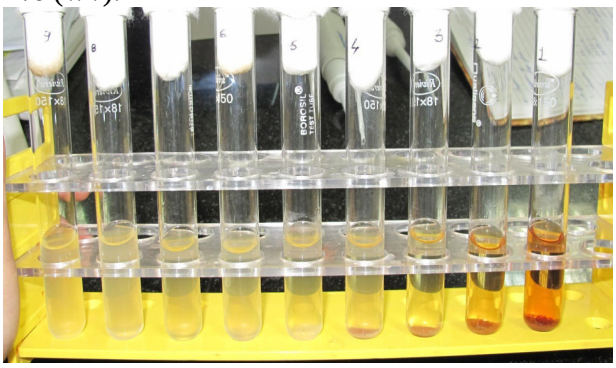

Fig.1 minimum inhibitory concentration study of Methanolic fraction against *Staphylococcus aureus*

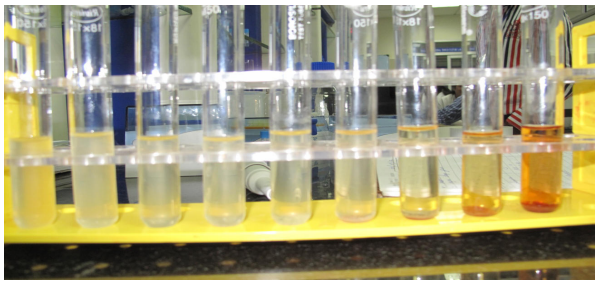

Fig2. Minimum inhibitory concentration study of N-Butenol fraction against *Staphylococcus aureus.*

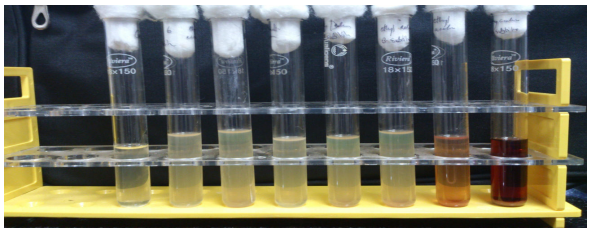

Fig 3. Minimum inhibitory concentration study of Methanolic fraction against *Bacillus subtilis*

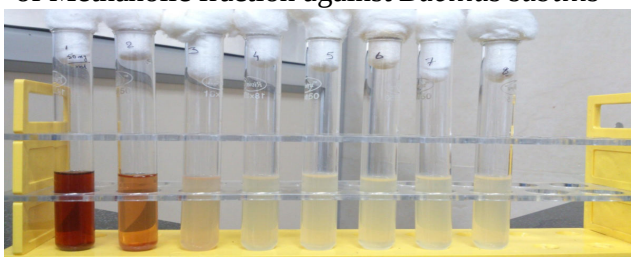

Fig 4. Minimum inhibitory concentration study of ethyl acetate fraction against *Staphylococcus aureus*

Table No. 8.1 Minimum Inhibitory Concentration

Bark of *Acacia Arabica*	Minimum inhibitory concentration	
	Bacterial strain	
	Staphylococcus aureus	***Bacillus subtilis***
Extract	**10 mg/ml**	**10 mg/ml**
Fraction 1	**25mg/ml**	**25mg/ml**
Fraction 2	**1mg/ml**	**25mg/ml**
Fraction 3	**0.5mg/ml**	**25mg/ml**
Streptomycin Drug	**0.1mg/ml**	**0.1mg/ml**

*Fraction 1; ethyl acetate Fraction 2; Methanolic Fraction 3; n-Butenol fraction

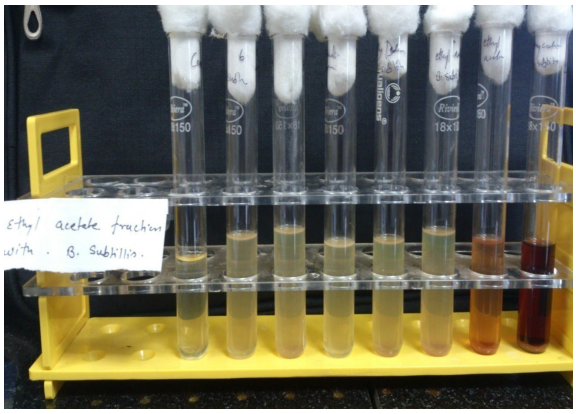

Fig 5. Minimum inhibitory concentration study of ethyl acetate fraction against *Bacillus subtilis*

Fig 6. Minimum inhibitory concentration study of N-Butenol fraction against *Bacillus subtilis*

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