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Antibacterial Screening and Phytochemical investigation of bark extracts of *Acacia jacquemontii* Benth.

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ABSTRACT

Acacia jacquemontii was assessed for active principles to ascertain the rationale for its use in traditional medicine. Preliminary phytochemical screening of the stem bark extracts showed that it possessed the active principles - alkaloids, glycosides, saponins, terpenoids and tannins. The antimicrobial activity of the extracts was assayed against pathogenic strains of *Bacillus cereus, Bacillus pumilus, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, S. pyrogenes, and Candida albcans* using the agar diffusion method. The plant extract exhibited antimicrobial activity against all the test microorganisms. *B. cereus* and *B. pumilus* were the most susceptible to the plant extract while *Candida albicans* was the most resistant. The minimum inhibitory concentration of the stem bark extract of the plant ranged between 30 and 50 mg/ml while the minimum bactericidal concentration ranged between 35 and 60 mg/ml. *A. jacquemontii* could be a potential source of antimicrobial agents.

Key words: Antibacterial, Antifungal, Baonli, Medicinal plants.

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 hollyback, these plants are still widely used in ethnomedicine around the world (Thomson, 1978; Stockwell 1988). The drugs contained in medicinal plants are known as active principles. The active principles are divided chemically into a number of groups among which are alkaloids, volatile essential oils, phenols and phenolic glycosides, resins, oleosins, steroids, tannins and terpenes (Mitcher et al., 1988; Habtermariam, 1993). Miski et al. (1983) reported the antibacterial activity of flavonoids from *Salvias palatine*. The authors identified ten aglycones and six glycosides of luteolin and apigenin from the leaf extracts. It was reported that cirsimaritin among the compounds identified showed antimicrobial activity against *Streptococcus aureus*, *Staphylococcus epidermitis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Pseudomonas aeruginosa.* Clark et al. (1981) showed that phenolic constituents of *Maynolia grandiflora* have some antibacterial activity. In an investigation carried out by Mitcher et al. (1988) on *Halianthus annuus*, some phenollics with antimicrobial property were isolated. 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 spectrum of its constituents.

Acacia jacquemontii Benth. locally called Bhu-baonli, Raati-baonli or Baonli, is a member of Fabaceae/Leguminosae. This family is incredibly diverse ranges from annuals to woody perennials. Legumes are simultaneously one of the largest families of crop plants and have been used in agricultural production since the earliest civilization. These have been used as food, feed,

forage, fiber, industrial and medicinal compounds, flowers and other end uses. In addition to traditional food and forage uses, legumes can be milled into flour, used to make bread, doughnuts, tortillas, chips, spreads, and extruded snacks or used in liquid form to produce milks, yoghurts and infant formula (Garcia et al. 1998). These have been used industrially to prepare biodegradable plastics (Pataeu et al. 1994), oils, gums, dyes and inks (Morris 1997). Many legumes have been used in folk medicine (Duke 1992; Kindscher 1992). *A. jacquemontii* is a rigid xerophytic shrub or small tree and characterized by zig-zag branches. This plant is distributed throughout semi-arid regions. A. jacquemontii is a versatile tree suitable for afforestation, social and agro-forestry. In addition to its normal utility in wood production, soil improvement, nitrogen fixation, it provide certain other products like fodder, fruits, gums, fibers and roofs. The bark of the root is used as inocula for fermentation and making local spirit. It produces dried gum on stem, which has demulcent properties and often added to medicine for this purpose (Al-Mosawi 2006). The powdered bark is also used induce spontaneous abortion, against snake bite as well as in various ethno-Medicare. Hence the aim of this study is to determine the phytochemical constituent and to investigate the antimicrobial properties of *A. jacquemontii* to ascertain the rationale for its use in traditional medicine.

MATERIALS AND METHODS

Collection of plant material

During the course of investigation several field surveys were conducted to collect the stem bark of tree. The survey was done in districts of Ajmer, Bikaner, Nagaur, Pali, Sikar, Churu and Jodhpur districts of Rajasthan, India.

Test microorganisms

Different group of pathogenic bacteria were isolated from various pathological samples from different hospitals of Jodhpur, India. The bacterial and microorganisms isolated and tested were *Bacillus cereus, Bacillus pumilus, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, S. pyrogenes and Candida albcans.*

Preparation of plant extract

Ethanolic extract of the stem bark of the plant was extracted according to the method described by Okogun (2000). A 50 g sample of the stem bark of the plant was air-dried, ground into powder using an electric blender. The blended material was transferred into a beaker and 10 ml of 95% ethanol was added at ambient temperature (28 ± 2°C). The mixture was extracted by agitation on a rotary shaker. Extraction was allowed to proceed for 48 h. The mixture was decanted and the solvent was removed by evaporation at room temperature (28 ± 2°C) to obtain the extract.

Phytochemical analysis of extract

The methods described by Harborne (1978) with slight modifications were used to test for the presence of the active ingredients in the test sample.

Test for alkaloids

The extract of the plant stem bark sample (0.5 g) was stirred with 5 ml of 1% HCl on a steam bath. The solution obtained was filtered and 1 ml of the filtrate was treated with two drops of Mayer's reagent. The two solutions were mixed and made up to 100 ml with distilled water. Turbidity of the extract filtrate on addition of Mayer's reagent was regarded as evidence for the presence of alkaloids in the extract (Harborne, 1978).

Test for flavonoids

A small piece of magnesium ribbon was added to ethanolic extract of the plant material, this was followed by the drop wise addition of concentrated hydrochloric acid. Colours varying from orange to red indicated flavones, red to crimson indicated flavonols, crimson to magenta indicated flavonones (Harborne, 1978).

Test for glycosides

Coarsely powdered stem bark (1 g) was added into two separate beakers. To one of the beakers was added 5 ml of dilute sulphuric acid while 5 ml of water was added to the other beaker. The two beakers were heated for 3 -5 min and the contents filtered into labeled test tubes. The filtrate was made alkaline with 5% sodium hydroxide and heated with Fehling's solution for 3 min. The

presence of reddish precipitate in the acid filtrate and the absence of such precipitate in the aqueous filtrate were regarded as positive for glycosides (Harborne, 1978).

Test for saponins
Stem bark of the test plant was ground into powder form and 0.5 g of the powdered stem bark was introduced into a tube containing 5.0 ml of distilled water, the mixture was vigorously shaken for 2 min, formation of froth indicated the presence of saponins.

Test for steroids
A 10 ml of chloroform extract of the test plant leaves was evaporated to a dry mass and the mass dissolved in 0.5 ml of chloroform. Acetic anhydride (0.5 ml) and 2 ml of concentrated sulphuric acid were added. A blue or green colour or a mixture of these two shades was regarded as positive for the presence of steroidal compounds.

Test for tannins
i.) 1 cm^3 of freshly prepared 10% KOH was added to 1 cm^3 of the extract. A dirty white precipitate indicated the presence of tannins.
ii.) Powdered stem bark of the test plant (1.0 g) was weighed into a beaker and 10 ml of distilled water added. The mixture was boiled for five minutes. Two drops of 5% $FeCl_3$ were then added. Production of greenish precipitate indicated the presence of tannins.

Test for terpenoids
The presence of terpenoids was determined as described for steroids except that red, pink or violet colour indicates the presence of terpenoids.

Antimicrobial assay
The antimicrobial assay was performed using the agar diffusion method of Collins et al. (1995). The test organisms were inoculated on nutrient agar plates and spread uniformly using a sterile glass spreader. Wells of 5 mm diameter were made on the nutrient agar using a sterile cork borer. The cut agar disks were carefully removed by the use of sterilized forceps. To each well was introduced various concentrations (10, 20, 30, 40, and 50 mg/ml) of the extracts. Control experiments comprising inoculum without plant extract were set up. The plates were allowed to stand for one hour at room temperature (25 ± 2°C) for diffusion of the substances to proceed before the growth of organisms commenced. The plates were incubated at 37°C for 24 h. The zones of inhibition were then recorded.

Determination of Minimum Inhibitory Concentration (MIC)
Various concentrations of the plant extract ranging between 10 and 60 mg/ml were introduced into different test tubes, each tube was inoculated with an overweight culture of *Bacillus cereus, Bacillus pumilus, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus pyrogenes and Candida albcans* were diluted to give a final concentration of 10^6 cells per ml. The tubes were incubated at 37°C for 24 h. The least concentration of the plant extract that did not permit any visible growth of the inoculated test organism in broth culture was regarded as the MIC in each case (Collins et al., 1995). Determination of minimum bactericidal concentration (MBC) After culturing the test organisms separately in nutrient broth containing various concentration of the stem bark extract of the plant, the broth was inoculated onto freshly prepared agar plates to assay for the bactericidal effect. The culture was incubated at 37°C for 24 h. The lowest concentration of the plant extract that does not yield any colony growth on the solid medium after the incubation period was regarded as MBC (Alade and Irobi, 1993).

RESULTS AND DISCUSSION

Phytochemical screening of the stem bark of *A. jacquemontii* revealed that the plant contain terpenoids, alkaloids, saponins and glycosides (Table 1). Negative results were recorded for steroids and flavonoids which confirm the absence of these active principles (Table 1).

The active principles identified in this study exhibited antimicrobial activity against all the test organisms (Table 2). Several plants, which are rich in alkaloids, tannins and glycol-sides, have been shown to possess antimicrobial activity against a number of microorganisms. For example, Adebajo et al. (1983) investigated the antimicrobial activity of leaf extract of *Eugenia uniflora* and

reported that tannins, glycosides and alkaloids were detected and that the ethyl acetate and methanolic leaf extract of the plant were active against *E. coli, P. vulgaris, K. pneumoniae* and *Aspergillus niger*. Saponins are a special class of glycol-sides which have soapy characteristic and facilitate the absorption of foods and medicine. Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional protein unavailable for them (Fluck, 1973). It therefore suggests that the medicinal plant used in the present study may have a general antimicrobial activity. The large size of the zones of inhibition indicated the potency of the active principles of the plant (Table 2). It was recorded that an increase in the concentration of the extract yielded higher activity as shown by the diameter of zone of inhibition (Table 2). The fact that organisms may need higher concentrations of extracts to inhibit or kill them may be due to their cell wall components.

Table 1: Phytochemical analysis of stem bark extract of *A. jacquemontii*

Extract	Alkaloid	Flavonoids	Glycosides	Saponins	Steroids	Tannins	Terpenoids
A. jacquemontii	+	-	+	+	-	+	+

+ = Present - = Absent

Table 2: Antimicrobial activities of stem bark extract on selected microorganisms

Conc. (mg/ml)	Mean diameter of zone of inhibition (mm) ± SD						
	BC	**BP**	**EC**	**PA**	**SA**	**SP**	**CA**
10	0	0	0	0	0	0	0
20	7.5 ±0.11	10±0.02	0	6.5±0.04	6.2±0.03	5.3±0.03	0
30	10.2±0.07	12±0.01	5±0.03	7±0.03	8.4±0.03	7.3±0.05	0
40	14.8±0.03	15±0.02	7.5±0.02	9±0.2	11.2±0.02	9.2±0.06	5.5±0.07
50	15.4±0.02	16±0.01	10±0.03	11±0.2	13.2±0.02	14.1 ±0.03	7.3±0.06

Bacillus cereus–BC, *Bacillus pumilus*-BP, *Escherichia coli*-EC, *Pseudomonas aeruginosa*-PA, *Staphylococcus aureus*-SA, *Staphylococcus pyrogenes*-SP, *Candida albcans*-CA.

The results (Fig 1) showed that the MIC of the stem bark extract of the plant ranged between 30 and 50 mg/ml. The effect of the plant extract on the MIC for the test microorganisms correlate with the report that microorganisms varied widely in the degree of their susceptibility (Emeruwa, 1982). Antimicrobial agents with a low activity against an organism have a high MIC while a highly active antimicrobial agent gives a low MIC.

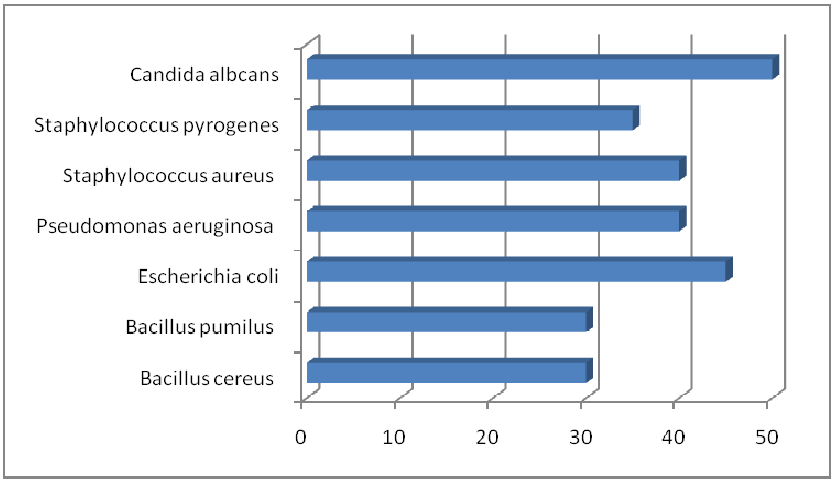

Figure 1: Minimum inhibitory concentration (MIC) of stem bark extract (mg/l) from *A. jacquemontii*.

The minimum bactericidal concentration (MBC) of the stem bark extract of the plant ranged between 35 and 60 mg/ml (Fig 2). The MIC and MBC which is normally used to evaluate the efficacy of the agents such as antiseptics, disinfectants and indeed chemotherapeutic agents (Croshaw, 1983) under standard conditions also support the sensitivity test results.

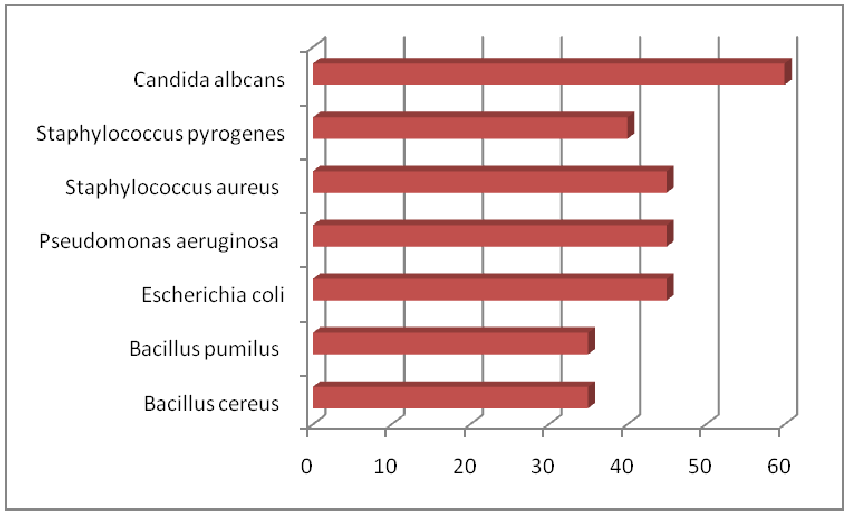

Figure 2: Minimum Bactericidal Concentration (MBC) of stem bark extract (mg/ml) from *A. jacquemontii*.

CONCLUSION

Results from present study shows that ethanolic stem bark extract of *Acacia jacquemontii* produced antimicrobial activity against various pathogens such as *Bacillus cereus, Bacillus pumilus, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus pyrogenes* and *Candida albcans*. The extract contains the active principles – terpenoids, tannins, alkaloids, saponins and glycosides. This study observes that *A. jacquemontii* has useful antimicrobial properties. Toxicological studies, purification and identification of the plant active principles should be embarked upon in addition to investigating its activity on a wider range of bacteria and fungi.

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