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EFFECTIVENESS OF MEDICINAL HERBS AGAINST DROMEDARY MASTITIS ISOLATES

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ABSTRACT

Screening of nine medicinal herbs viz, Tulsi (*Ocimum sanctum*), Ashwagandha (*Withania somnifera*), Datura (*Datura metel*), Peepal (*Ficus religiosa*), Pardesi Kiker (*Prosopis juliflora*), Anar (*Punica granatum*) leaves, Garlic (*Allium sativum*) bulb, Karela (*Momordica charantia*) fruit, Ginger (*Zingiber officinale)* root for antibacterial activity against 76 bacterial isolates from camel intramammary infections, which comprised of *Staphylococcus (St.) epidermidis (*34), *St. aureus (*16), *Corynebacterium* spp *(*9), *Micrococcus* spp (4), *Bacillus* spp *(*5) and *Escherichia coli* (8) revealed 100 percent sensitivity against crude and methanol extract of anar and pardesi kiker leaves. Datura, ashawagandha and garlic were also found to possess good antibacterial activity, whereas crude juice of peepal exhibited 100 per cent activity against *E.coli* isolates. On exposure of methanolic extract of these plants to UV rays antibacterial activity of kiker and anar was unaffected whereas all other plants failed to show any antibacterial activity. *In vitro* MIC of methanol extract of anar leaves varied from 3.75 to 10 µl/ml and for pardesi kiker from 1.25 to 8.75 µl/ml. No significant synergistic effect was observed by combining two plants extracts.

Key words: Camel, mastitis, medicinal herbs

Mastitis is internationally recognised as one of the most important animal diseases. A total of 137 species, subspecies and serovarities of organisms have been isolated from the mammary gland (Watts, 1988). Since the introduction of antibiotics, there has been tremendous increase in the resistance of diverse bacterial pathogens. Plant derived products have been used for medicinal purposes for centuries and they possess an almost limitless ability to synthesise aromatic substances such as flavanoids (Geissman, 1963). Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10 per cent of the total (Schultes, 1978). In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects and herbivores. Many laboratories have found literally thousands of phytochemicals, which have inhibitory effects on all types of microorganisms in vitro. More of these compounds should be subjected to animal and human studies to determine their effectiveness in whole organisms systems.

The present work was carried out to elucidate the antibacterial action of some of the commonly available medicinal plants and herbs against microorganisms causing intramammary infections in the dromedary.

Materials and Methods

A total of 76 bacterial isolates from camel intramammary infections, which comprised of *St. epidermidis (*34), *St. aureus (*16), *Corynebacterium* spp *(*9), *Micrococcus* spp (4), *Bacillus* spp *(*5) and *Escherichia coli* (8) were selected to investigate the antibacterial activity to crude juice and methanol extract of Tulsi (*Ocimum sanctum*), Ashwagandha (*Withania somnifera*), Datura (*Datura metel*), Peepal (*Ficus religiosa*), Pardesi Kiker (*Prosopis juliflora*), Anar (*Punica granatum*) leaves, Garlic (*Allium sativum*) bulb, Karela (*Momordica charantia*) fruit, and Ginger (*Zingiber officinale)* root.

Source of herbs

Traditional medicinal herbs viz *Ocimum sanctum*, *Withania somnifera, Datura metel, Ficus religiosa*, *Punica granatum* and *Prosopis juliflora* leaves were collected locally. Vegetable herbs viz. *Allium sativum*, *Momordica charantia* and *Zingiber officinale* were procured from the local market. All these herbs were identified by a botanist.

Extraction of crude juice

Juices of fresh herbal plants were extracted with a juicer-mixer (Phillips India Limited Home use type) in the laboratory. These juices were filtered

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through a Whatman filter paper no 1 (Qualigens) and then scitz filtered. These juices were stored at 4°C till further use.

Methanol extraction

All herbs were either cut into small pieces or as such were dried under a shade and were coarse grounded. Five grams of the coarse powder were mixed with 100 ml of methanol in glass stoppered bottles which were kept overnight at room temperature. The following day, these mixtures were vortexed for 10 minutes and then centrifuged at 3000 rpm for 10 minutes. The resulting supernatant of each herb was collected separately in glass beakers and chilled in a freezer for 2 hours. Thereafter, the liquid portion was poured into a clean separate beaker and the methanol evaporated at 37-40°C. The final volume was reconstituted to 5 millilitre normal saline and was stored in refrigerator till further use.

Antibacterial activity

For conducting antibacterial sensitivity tests 2-3 pure single colonies of fresh cultures were suspended in 3 ml of sterilised nutrient broth and were incubated at 37°C for appearance of turbidity. These cultures were then spread over nutrient agar plates with cotton swabs under sterilised conditions. For each culture, 3 plates were used and each plate was divided into 6 parts. The parts are marked as follows:

Plate 1: CT= crude tulsi, MT=methanol tulsi, CAg= crude ashwagandha, MAg = methanol ashwagandha, CD = crude datura, MD = methanol datura.

Plate 2: CP= crude Peepal, MP= methanol Peepal, CA= crude anar, MA = methanol anar, CK = crude kiker, MK = methanol kiker.

Plate 3: CGr= crude garlic, MGr= methanol garlic, CKr= crude karela, MKr = methanol karela, CGn = crude ginger, MGn= methanol ginger.

Into the centre of each part, a well was punched and 10 ml of either crude juice or methanol extract was pipetted into the wells. Plates were kept at room temperature for one hour to facilitate diffusion and were then incubated at 37°C for 24 hours. The inhibition diameter was measured. Results were interpreted as positive when the diameter of the inhibition zone was more than 10 mm. The procedure for antibacterial sensitivity was carried out according to Novarro *et al* (1996).

Ultraviolet rays treatment of methanol extract

The methanol extracts of each plant were exposed to UV rays in a laminar flow (15Wx40 minutes) as 1 mm thick layer, and these extracts were tested for antibacterial activity against 6 isolates comprising *St. aureus* (5), *E. coli* (2) and *Pseudomonas* spp (1) to screen for the presence of photosensitive phytochemicals.

Synergistic effect

To investigate the synergistic effect, the methanol extracts of two plants were mixed. All possible combinations were tested for all plants in 1:1 ratio (5µl+5µl) on 6 isolates comprising *St. aureus* (5), *E. coli* (2) and *Pseudomonas* spp. (1).

In vitro minimum inhibitory concentrations

Methanol extracts of anar (*Punica granatum)* and pardesi kiker *(Prosopis juliflora*) were evaluated *in vitro* for MIC against 6 isolates comprising *St. aureus* (5), *E. coli* (2) and *Pseudomonas* spp (1) in nutrient broth by dilution method.

Table 1. Antibacterial sensitivity of crude and methanol extract of medicinal herbs.

Bacterial	No of isolates found sensitive																		
	No of isolates	Tulsi leaves		Ashwagandha Leaves		Datura Leaves		Peepal Leaves		Anar Leaves		Kiker Leaves		Garlic bulb		Karela Fruit		Ginger Root	
		CT	MT	CAg	MAg	CD	MD	CP	MP	CA	MA	CK	MK	CGr	MGr	CKr	MKr	CGn	Gn
St. epidermidis	34	12	1	34	30	34	34	0	1	34	34	34	34	28	8	13	7	11	0
St. aureus	16	3	0	16	16	15	15	0	0	16	16	16	16	16	1	2	1	1	0
Corynebacterium spp.	9	6	1	9	9	9	9	0	0	9	9	9	9	9	1	6	4	2	1
Micrococcus spp.	4	2	0	4	4	4	4	0	0	4	4	4	4	4	0	3	2	0	0
Bacillus spp.	5	4	1	5	5	5	5	0	0	5	5	5	5	5	1	4	2	4	1
E. coli	8	0	0	0	0	0	0	8	0	8	8	8	8	8	1	0	0	0	
Total	76	27	3	68	64	65	67	8	1	76	76	76	76	70	12	28	16	18	2
Overall percentage		35.52	3.95	89.47	84.21	85.53	88.16	10.52	1.31	100	100	100	100	92.10	15.78	36.84	21.05	23.68	2.63

Results

Screening of nine medicinal herbs revealed 100 per cent sensitivity against crude and methanol extracts of anar and pardesi kiker leaves. Datura, ashawagandha and garlic were also found to possess a good antibacterial activity, whereas crude juice of peepal exhibited 100 per cent activity against *E.coli* isolates (Table 1).

Antibacterial activity after exposure to UV

Antibacterial activity of kiker and anar was unaffected by UV treated methanol extract, whereas all other plants failed to show any antibacterial activity (Table 2).

Synergistic effect

No significant synergistic effect was observed by combining two plants extracts with any combination. All these combinations gave almost comparable zone of inhibition as observed with single plant extract.

Comparison of antibacterial effects of plants with standard antibiotics

Antibacterial sensitivity results against 24 bacterial isolates comprising *St. aureus* (16), *St. epidermidis* (4), *E.coli* (2) and *Pseudomonas* spp (1) were compared with the antibacterial effect of various plant extracts. The results revealed almost comparable effects of 10 µl anar and pardesi kiker extracts with tetracycline sensitivity, when a diameter inhibition zone of 10 mm was considered sensitive for these plants. Cloxacillin gave poor sensitivity against *St. aureus* isolates. Four isolates of *St. aureus* which were resistant to cloxacillin were found sensitive with anar and pardesi kiker extracts.

Table 2. Antibacterial activity after exposure to UV rays.

Plant	Organism (no of isolates)			Total (8)
	St. aureus (5)	*E. coli* (2)	*Pseudomonas* spp. (1)	
	No of isolates found sensitive			
Datura leaves	-	-	-	-
Anar leaves	5	2	1	8
Peepal leaves	-	-	-	-
Kiker leaves	5	2	1	8
Tulsi leaves	-	-	-	-
Ashawagandha leaves	-	-	-	-
Garlic bulb	-	-	-	-
Karela fruit	-	-	-	-
Ginger root	-	-	-	-

In vitro minimum inhibitory concentrations

MIC of methanol extracts of anar leaves varied from 3.75 to 10 µl/ml and for pardesi kiker from 1.25 to 8.75 µl/ml.

Discussion

The development of antibiotic resistance is a common problem with most of the infections. While treating mastitis with antibiotics in developing countries especially in unorganised herds, the milk withholding time recommended for human consumption is not strictly followed thereby it may further aggravate the development of resistant bacterial strains. Since most of plants are part of the food chain is either being consumed directly by the human being or by the animals, therefore, the present study is oriented towards development of antibiotic replacement treatment for mastitis from plants.

Ocimum sanctum has versatile role in traditional medicine. In the present study, some antibacterial activity against *St. aureus* and no activity against *E. coli* were observed with crude extract of *Ocimum sanctum*. Gupta *et al* (2002) observed almost similar trend against these organisms with aqueous leaf extract of *Ocimum sanctum*. Higher content of linolenic acid in *Ocimum sanctum* fixed oil could contribute towards its antibacterial activity (Singh *et al,* 2005). Malondialdehyde is an aldehyde formed as a breakdown product of peroxidised polyunsaturated lipids (Hall *et al*, 1997). Therefore, linoleic acid and linolenic acid on oxidation could give malondialdehyde. Malondialdehyde is a cross linker and initiates oxidation reaction in which undesirable bonds form between nucleic acids (Leuzaj and Skrzydlewska, 2003). The probable result is inhibition of DNA replication. In addition, malondialdehyde could also crosslink amino group of bacterial enzymes and thereby inhibit the growth. The therapeutic efficacy of *Ocimum sanctum* fixed oil against mastitis suggests that it has the potential to replace the steroid (if not both antibiotic and steroid) and offer a cheaper therapy for the disease (Singh *et al,* 1995).

Potent antibacterial activity exhibited by withania against staphylococcus organisms is in comparision to but no activity against *E.coli* is contrary to findings of Abdulmoniem *et al* (2006); they reported extracts from *Withania somenifera* active against some G +ve and G -ve pathogenic bacteria. Medically withania leaves are used internally for fever and haemorrhoids and externally for wounds, haemorrhoids, tumours, tuberculous glands, anthrax

pustules, syphilitic sores, erysipelas, and ophthalmitis (Kirtikar and Basu, 1991; Varrier, 1996).

Datura medically has a wide range of applications, including in the treatment of epilepsy, hysteria, insanity, heart diseases, fever with catarrh, diarrhoea, skin diseases etc (Chopra *et al,* 1986). Obi *et al* (2001) reported marked antibacterial activities with the roots and stems of *Datura stramonium* against G+ve and G-ve bacteria of medical importance. Similar activity against G+ organisms was observed in the present investigation with *Datura metel* leaves.

Potent antibacterial activity against all isolates of *E.coli* tested was shown by *Ficus religiosa* leaves. Odunbaku *et al* (2008) reported ethanolic leaf extract of *Ficus exasperata* showed a MIC of 300 mg/ml for *E.coli.* Extracts of *Ficus religiosa* leaves also demonstrated some antibacterial activity (Farrukh and Iqbal, 2003).

Significant antibacterial activity against all the test organisms was observed with *Punica granatum* leaves. Negi and Jayaprakasha (2003) reported antioxidant and antibacterial activities of *Punica granatum* peel extracts. The aqueous extract of leaf of punica was able to inhibit the growth of *Bacillus subtilis* and *St. aureus* (Nair and Chanda, 2005).

In the present study, significant antibacterial activity observed with *Prosopis Juliflora* support the findings of Aqeel (1991). Antimicrobial activity of the juliflorine, julifloricine and a benzene insoluble alkaloidal fraction isolated from *Prosopis juliflora* were found to posses remarkable effect against G +ve bacteria as well as G -ve *Campylobacter* spp. Qazi (1987) found Juliflorine more effective than penicillin, sulphamethoxazole and erythromycin against *St. aureus*. Caceres *et al* (1995) studied plants popularly used in Guatemala for the treatment of gonorrhoea and found *Prosopis juliflora* to posses *in vitro* activity against *Neisseria gonorrhoeae* using strains isolated from symptomatic patients.

Good antibacterial activity against G+ve bacteria and 100 percent against *E.coli* isolates with *Allium sativum* supports the therapeutic efficacy against *E. coli* infection in chickens (Rao *et al,* 1983). Indu *et al* (2006) showed excellent antibacterial activity at all concentrations (100%, 75%, 50% and 25%) of garlic extract to various serogroups of *E. coli* tested. Siegers *et al* (1999) suggested that the alliin metabolite allicin might be responsible for the oxygen scavenging properties of *Allium sativum.* It is postulated that the antibacterial and antifungal properties of garlic juice are due to the inhibition of succinic dehydrogenase via the inactivation of thiol group.

Leaf extracts of *Momordica Charantia* have clinically demonstrated broad-spectrum antimicrobial activity. Various water, ethanol, and methanol extracts of the leaves have demonstrated *in vitro* antibacterial activities against *E. coli*, Staphylococcus, Pseudomonas, Salmonella, Streptobacillus and Streptococcus (Omoregbe *et al*, 1996 and Khan and Omoloso, 1998). In another study, a fruit extract has demonstrated activity against the stomach ulcer-causing bacteria *Helicobacter pylori* (Yesilada *et al*, 1999). In this study it failed to show antibacterial activity against *E. coli* tested.

Zingiber officinale was not found sensitive against *E.coli* tested, which is contrary to the findings of Cohen (1992) that ginger extract had moderate antibacterial properties against *E. coli* serogroups O^{8} and O^{88}. Ekwenye and Elegalam (2005) observed that the solvent of extraction affected the degree of antibacterial activity of the extracts. It was observed that the ethanolic extract of ginger gave the widest zone of inhibition (22 mm) using the concentration of 0.8 g ml^{-1}

High antibacterial activity observed in some of the plants with crude extract compared to methanol extract suggests that either all the active components are not soluble in methanol or some of the compounds get degraded with storage.

In the present study, no significant synergistic effect was observed by combining methanol extract of two plants. Sensitivity with standard antibiotics revealed comparable effects of 10 µl anar and pardesi kiker extracts with tetracycline sensitivity, whereas cloxacillin gave poor sensitivity against *St. aureus* isolates. Even 4 isolates of *St. aureus,* which were resistant to cloxacillin, were found sensitive with anar and pardesi kiker extracts.

Benefits of phytomedicines often result from synergistic actions of multiple active chemicals acting at single or multiple target sites associated with a physiological process. This synergistic or additive pharmacological effect can be beneficial by eliminating the problematic side effects associated with the predominance of a single xenobiotic compound in the body (Tyler, 1999). Multiple chemicals acting in an additive or synergistic manner likely has its origin in the functional role of secondary products in promoting plant survival (Kaufman *et al*, 1999). The role of secondary products as defense chemicals, a mixture of chemicals having additive or synergistic effects at multiple target sites would not only ensure effectiveness against a wide range of herbivores or

pathogens but would also decrease the chances of these organisms developing resistance or adaptive responses (Kaufman *et al*, 1999 and Wink, 1999). Secondary products involved in plant defense through cytotoxicity toward microbial pathogens could prove useful as antimicrobial medicines for animals, if not too toxic.

Further antibacterial activity against pathogenic organisms like *St. aureus, E. coli* and *Pseudomonas* spp observed with *Prosopis juliflora* and *Punica granatum* may be of value for considering these plants for the treatment of certain infectious diseases, after evaluation of cytotoxicity, storage stability and excretion of the compounds after degradation or as such from the body.

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