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Antimicrobial Activity of Leaf Extracts of *Murraya Koenigii* against Aerobic Bacteria Associated with Bovine Mastitis

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ABSTRACT

The susceptibilities of some of the common aerobic bacterial isolates from 20 milk samples collected from different cows with udder inflammation were subjected to hexane and methanolic leaf extracts of *Murraya koenigii* was determined by the well diffusion method. The minimum inhibitory concentration (MIC) of test extracts that gave inhibition were determined using the tube dilution method. The phytochemicals present in the various leaf extracts were also qualitatively assayed using conventional techniques. The methanolic extract of leaves of *Murraya koenigii* inhibited *Staphylococcus aureus*, *Staphylococcus epidermidis, Streptococcus uberis, Pseudomonas aeruginosa, Escherichia coli, Corynebacterium gravis* and *Bacillus cereus*. The hexane extract inhibited all microorganisms except *Staphylococcus epidermidis*, *Streptococcus uberis* and *Bacillus cereus*. The MIC values of the different methanolic extracts of leaves were found to vary greatly, and ranged from 8.25 mg/ml to 30mg/ml.

Key words: Bovine mastitis, aerobic bacteria, *Murraya koenigii*,methanolic extracts.

INTRODUCTION

Bovine mastitis is the inflammation of the parenchyma of the mammary glands of cattle [7] associated with microbial infections [8] and physiological changes[9]. Mastitis is caused by a group of infective and potentially pathogenic bacteria [3], viruses [11], mycoplasma , fungi and algae. The most common bacteria causing bovine mastitis include; *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus uberis*, *Streptococcus dysgalactia* and *Escherichia coli*. In order to minimize the economic losses from bovine mastitis and dissipation of infection resulting from the consumption of contaminated milk and milk products, there is an urgent need to ascertain the current status and involvement of aerobic bacteria in bovine udder inflammation, their role as causative agents of bovine mastitis and their susceptibilities**.**

MATERIALS AND METHODS

Collection of plant material

Fresh leaves of *Murraya Koenigii* were collected from the local gardens and were rinsed properly in sterile distilled water, dried in shadow and then ground to powdered form. The leaf powder was then extracted using methanol and hexane as solvents with the soxhlet apparatus. The extracts so obtained were weighed and stored in sterile universal bottles at 4 ^{0}C in a refrigerator.

Fig.-1: Plant of *Murraya Koenigii*

Phytochemical Screening
The methanolic extract, aqueous extract and leaf powder of *Murraya koenigii* were used as samples for qualitative phytochemical screening for tannins, resins, alkaloids, saponins, tannins, glycosides and flavonoids following the standard procedures described by Trease and Evans and Faraz [6,10].

Test for resins:
To 0.5g of each sample was added 5ml of boiling ethanol. This was filtered through Whatman No.1 filter paper and the filtrate diluted with 4ml of 1% aqueous HCl. The formation of a heavy resinous precipitate indicated the presence of resins [10]. This was filtered and 1ml of the filtrate tested with a few drops of Dragendorff's reagent and a second 1ml portion treated similarly with Wagners reagent. The formation of a precipitate was an indication of the presence of alkaloids [6].

Test For Saponins:
0.5g of each sample was stirred with water in a test tube. Frothing which persists on warming was taken as preliminary evidence for the presence of saponins [6].

Test For Tannins:
0.5g of each sample was stirred with 10ml of boiling distilled water. This was filtered and a few milliliters of 6% ferric chloride added to the filtrate. Appearance of deep green coloration indicated the presence of tannins. The second portion of the filtrate was treated with a few milliliters of iodine solution. Appearance of a faint bluish coloration confirmed the presence of tannins [10].

Test For Glycosides:
0.5g of each sample was stirred with 10ml of boiling distilled water. This was filtered and 2ml of the filtrate hydrolized with a few drops of concentrated HCl and the solution rendered alkaline with a few drops of ammonia solution. 5 drops of this solution was added to 2ml of Benedict`s qualitative reagent and boiled. Appearance of reddish brown precipitate showed the presence of glycosides [10]

Test For Flavonoids:
0.5g of each sample was dissolved in 2ml dilute NaOH solution. A few drops of concentrated sulphuric acid was then added. The presence of flavonoids was indicated by the disappearance of color [10].

Isolation and Identification of Bacteria
20 samples (5ml each) of cow milk was aseptically collected from each quarter of the udders of 20 lactating cows into sterile sample bottles labeled with the number of the animal following the method of Schalm *et al* [12]. Each sample was examined macroscopically for discoloration clots and flakes. Somatic cell counts were done using the manual method. Each sample was then inoculated in duplicate onto 10% sheep blood agar plates and were incubated aerobically at 37^0C for 48 hours and were examined for growth over a period of 24 and 48 hours respectively. Cultures were then identified using conventional techniques [2].

Sensitivity Test
Colonies of fresh cultures were suspended in 20 ml of nutrient broth in different sterile universal bottles and were incubated at 37^0C over night. The concentration of organisms in the broth was determined and was diluted down to 10-6 Macfarland's standard. One ml of this was used in flooding over nutrient agar plates in the well diffusion method of the *in vitro* antimicrobial sensitivity test. The plates were left for 5mins after which they were dried at 37 ^{0}Cfor 1hour. Four wells, equally distant, were bored round the plate using a sterile cork borer. Various concentrations of the diluted extracts were put inside the wells. Solvents such as Methanol and Hexane were put inside the well in separate petriplates to serve as negative control while Chloramphenicol(1mg\ml) was used as positive control in the separate petriplates. The plates were left free for 1 hour after which there were incubated at 37 ^{0}C for 24 hours and were examined for zones of inhibition.

Minimum Inhibitory Concentration (MIC)
The broth dilution assay was carried for different dilutions of the extracts using susceptible bacteria as described by Muray *et al*.[13].

RESULTS AND DISCUSSION

The phytochemical analysis of the leaf powder and various extracts gave the results as depicted in Table-1. Out of the 20 samples analysed, 18 had somatic cell counts (SCC) greater than $5x10^5$ cells/ml. Sixteen out of these 18 samples gave positive cultures. Hence, 16 samples out of 20 were positive. *Staphylococcus aureus* was the predominant organism isolated with 5 isolates. Others were *Streptococcus uberis* and *Corynebacterium gravis* with 3 isolates each; *Staphylococcus epidermidis* with 2 isolates; *Escherichia coli*, *Bacillus cereus* and *Pseudomonas aeruginosa* with 1 isolate each. 2 samples gave a mixed culture of *Staphylococcus aureus* and *Corynebacterium gravis*(Table-2). Results obtained from the susceptibility testing of the organisms with the various extracts showed that the methanolic extract was the potent antimicrobial agent than hexane extract. Methanolic extract inhibited *Staphylococcus aureus*, *Staphylococcus epidermidis, Streptococcus uberis, Pseudomonas aeruginosa, Escherichia coli, Corynebacterium gravis* and *Bacillus cereus*, while the hexane extract only inhibited *Pseudomonas aeruginosa*, *Escherichia coli* and *Corynebacterium gravis* (Table-3). Methanolic extract was found to be most potent antimicrobial agent against *Staphylococcus epidermidis* while hexane extract showed maximum antimicrobial activity against *E.coli* amongst all other microorganisms(Table-3). According to results obtained from MIC, the methanolic extract gave a MIC of 8.25mg/ml for *Escherichia coli* and S*taphylococcus epidermidis* and *Bacillus cereus* while 12.5 mg/ml for *Staphylococcus aureus* and *Streptococcus uberis.* Methanolic extract gave MIC of 30 mg\ml for *Pseudomonas aeruginosa* and *Corynebacterium gravis*(Table-4).

Table-1: Phytochemical analysis of *Murraya koenigii* leaves extracts

(+ means Present, ++ means Prominent, - means Absent)

Test	Hexane extracts	Methanolic extracts
Steroids	+	++
Alkaloids	++	++
Saponins	++	++
Cardiac Glycosides	+=	+
Reducing sugars	-	-
Flavonoides	++	++
Tannins	-	-

Table-2: Bacterial Isolates

Number of Cattle selected	Milk samples	Sample with SCC	Positive Samples	Bacteria isolated	Number of Pure isolates
20	20	18	16	*Staphylococcus aureus*	5
				Staphylococcus epidermidis	2
				Streptococcus uberis	3
				Escherichia coli	1
				Pseudomonas aeruginosa	1
				Corynebacterium gravis	3
				Bacillus cereus	16

Normal milk should contain less than 500,000 cells/ml and an elevated somatic cell count is an indication of inflammation in the udder [8]. As such elevated somatic cell counts in the present work was an indication of inflammation. Institute for Animal Health (2003) identifies all the organisms isolated during present work as causative agents of bovine mastitis and have produced experimental infections in cattle with these organisms. The inhibition of *Staphylococcus epidermidis, Streptococcus uberis, Pseudomonas aeruginosa, Escherichia coli, Corynebacterium gravis* and *Bacillus cereus* with an MIC range of 8.25 to 30 mg/ml suggest that *Murraya koenigii* stands as a promising alternate source of antibacterial agents for the management of diseases of animals however, further work is needed to refine the technique.

Table-3: Antibacterial Activity of Leaf extracts of *Murraya koenigii* and diameter of zone of inhibition (mm)

Bacteria	Methanolic extract (mg/ml)			Hexane Extract (mg/ml)			Chloramphenicol (mg/ml)
	200	100	50	200	100	50	
Staphylococcus aureus	20.00	18.00	12.00	18.00	15.00	12.00	25.00
Staphylococcus epidermidis	30.00	25.50	19.00	0.00	0.00	0.00	23.00
Streptococcus uberis	20.50	15.30	12.00	0.00	0.00	0.00	26.00
Pseudomonas aeruginosa	16.00	14.00	12.00	13.00	11.00	9.00	26.00
Escherichia coli	26.00	22.00	19.00	14.00	12.00	11.00	25.00
Corynebacterium gravis	15.00	14.00	12.00	13.00	12.00	12.00	28.00
Bacillus cereus	22.00	17.00	13.00	0.00	0.00	0.00	20.00

Table-4: Minimum Inhibitory concentration(MIC) of Methanolic extract of *Murraya koenigii*

Bacteria	Methanolic extract MIC(mg\ml)
Staphylococcus aureus	12.5
Staphylococcus epidermidis	8.25
Streptococcus uberis	12.5
Pseudomonas aeruginosa	30
Escherichia coli	8.25
Corynebacterium gravis	30
Bacillus cereus	8.25

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