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Antidiarrheal and Antimicrobial Activities of Ethno Medicinal Plant: *Mikania scandens* (L) Willd

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Original Research Article

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Abstract: The aim of the present study was carried out to investigate the antidiarrheal and antimicrobial potential of both ethanol and aqueous extracts of aerial parts of *Mikania scandens .In vivo* Antidiarrheal activity of *M.scandens* ethanol and aqueous aerial extracts at a dose of 100 mg/kg and 200 mg/kg BW were studied using experimentally induced castor oil diarrheal in Wister rats of both sexes and compared to standard drug loperamide (3mg/kg p.o). During this study different parameters like time of onset of diarrhoea, total nos. of faecal output (frequency of defecation) and weight of wet faeces, nos. of wet faeces were assessed. *In vitro* Antimicrobial activity (zone of inhibition, minimum inhibitory concentration, minimum bactericidal concentration, minimum fungal concentration) were performed by using four bacterial strains.(gram positive, & gram negative) and two fungal strains. Data were analysed using one way ANOVA. (Analysis of Variance).The results of antidiarrheal studies showed that over four hours, both the extracts significantly protected ($p<0.05$) the rats against castor oil induce diarrhoea, inhibited gastrointestinal transit, reduced the frequency of defecation in the treated groups compared with the untreated group. The crude ethanol extracts also showed good antimicrobial activity against all tested organisms. The obtained results allow justifying the traditional uses of *Mikania scandens* possess good antidiarrheal and antimicrobial activities .Results of the present study has revealed credence to the traditional usage of this plant as antidiarrheal and antimicrobial agent.
Key words: Antidiarrhoeal activity, Antimicrobial activity, Agar well diffusion method, *Mikania scandens,* loperamide.

INTRODUCTION

Since eternity nature has gifted us abundant of herbs and plants which form the main source of herbal preparation for therapy of numerous ailments and are still extensively used. In many developing countries, traditional medicine is one of the primary health care systems [1] because of these are unimpeded, toxicant, beneficial and reliable, easy availability. Medicinal plants constitute the utmost component of the traditional medicine practiced worldwide due to the economic viability, accessibility and ancestral experience [2].

Diarrhoea is one of the water borne infectious disease denoted by increased frequency liquidity of bowel movement, fluidity of the wet stool, and abdominal pain. It is an impaired absorption and hyper secretion syndrome of the gastrointestinal tract [3]. According to the World health organisation, it is the second leading cause of mortality and morbidity throughout the world effecting mostly the infants and children's. An estimated 88% of diarrhoeal related disease originated due to poor sanitation, non-hygienic condition, and malnutrition in developing countries. It may be brought about by viruses, bacteria, protozoa, drugs and bacteria endotoxins [4]. The major causative organisms of diarrhoea are *Staphylococcus aureus, Escherichia coli, Salmonella typhi* [5] and *candida albicans.* [6]. WHO has supported studies for treatment and prevention of diarrhoeal diseases depending on traditional medical practices.

Communicable, infectious diseases are the leading cause of death worldwide. Phytochemical evaluation of plants showed the presence of highly active biodynamic compounds can serve valuable source of natural antibacterial agents. The different traditional medicinal plant extracts has led to potential source of new antimicrobial agents [7].

Mikania scandens (L) Willd (Asteraceae) is also known as climbing hemp weed or climbing Boneset. It is traditionally used in the treatment of ulceritis, analgesic, and anti-inflammatory, anthelmintic. The ethno botanical uses of the *M.scandens* are to treat itchy skin, also for

circumcision wounds and tumours, planted as an ornamental, cover crop, and cattle feed [8-9]. Therefore it is required to accept the scientific basis for the therapeutic actions of traditional plant medicines as these may serve as the source for the development of more potent drugs.

The aim of the present study was to investigate the possible anti diarrhoeal (*in vivo*) and antimicrobial (*in vitro*) properties of the aerial extracts of *Mikania scandens* in order to establish the claimed biological activities of ethanol and aqueous extracts of this plant.

MATERIALS AND METHODS

Plant material

Aerial parts of *Mikania scandens* (leaves, stem, flowers) collected during June august 2014 from rural areas of Midnapore district of West Bengal, India. Identity of the plant was confirmed by Scientist-"O"V.P Prasad, taxonomist at the Central National Herbarium, Botanical garden, Howrah (Voucher No.CNH/57/2014/tech II/278) .The fresh aerial parts collected in bulk form and dried at room temperature under shade and then grind to coarse powder.

Preparation of the Extracts

The powder materials were loaded into Soxhlet apparatus and extracted with petroleum ether (for removing of fatty substances) chloroform and ethanol and then aqueous extracts were done by maceration successively. The extracts were filtered using whatmann no. 1 filter paper and concentrated using rotary evaporator and stored at a freezer in airtight screw capped bottle until further use.

Preliminary Phytochemical Analysis

Both the extracts were tested for the phytochemical evaluation [10-11]. Flavonoids ($FeCl_3$ and shinoda test) Alkaloids (dragendroff's and meyers reagent).Saponins with ability to produce stable foam, steroids (Lieberman buchard reagent) glycosides (brontrager's and Keller killiani test) Tannins and phenolic compound(5% $Fecl_3$ test and lead acetate).

Experimental Animals

Thirty six healthy Wister albino mice of either sex weighing 25-30 gm were selected for this study. The animals were accommodated to be acclimatising for two weeks before being subjected to experimental protocol. All the animals were housed in polypropylene cages and maintained under standard laboratory of temperature conditions in standard housing conditions at an ambient temperature of (25º±2) with 35-60% relative humidity and12/12 hour's light/dark cycle [12]. Each group of animals (n=6) had free access to water ad libitum a normal diet (Hindustan Unilever).All the test were performed according to OECD guidelines and institutional Animal Ethical Committee (IAEC) approved the study protocol (IAE/SKIPS/2014/OCT06/04/RATS-72/MICE-72) Committee for the Purpose of Control and Supervision of Experimental Animal (CPCSEA).

Test strains

Two gram positive bacteria *Bacillus subtilis* (MTCC No. 441), *Staphylococcus aureus* (MTCC No.3160) ,two gram negative bacteria *Escherichia coli* (MTCC No. 1652) *Salmonella typhi* (MTCC No. 733) and two fungal strains, *Candida albicans* (MTCC No.227), *Asperigillus niger* (MTCC No. 282) were obtained from microbiology department, which were kept at 4ºc on agar slant and subculture at 37ºc for 24 hrs. on nutrient agar before any susceptibility test.

Acute toxicity test

The acute toxicity study of both extracts were done as per OECD 420 guidelines [13] for different doses between 1000 and 2000 gm/kg orally in mice by gavage. This test was performed three times on a single test animal as a part of an oral Acute Toxicity Assay [14]. Healthy adult albino mice weighing between 25 to 35 gm were used for this study. After oral administration of both extracts various parameters body temperature, CNS activity, micturation, defecation etc were observed for 24 hrs.Accordingly100 and 200 mg/kg were selected for treatment during the study.

Castor oil induced diarrhoea

The method was designed by Shoba and Thomas [15] for the study of intestinal fluid secretion, gastrointestinal transit. The animals were screened initially by giving 0.5 ml of castor oil one week before the actual experiment. Only those producing diarrhoea were selected for final experiment. The rats were divided into six groups (n=6) and they were kept for overnight fasting with free access of water before experiment.

Table-1: Groups of Treatment

Serial No.	Groups
i)	Control (Distilled water with 0.5ml castor oil p.o.).
ii)	Standard (Loperamide 3mg/kg with 0.5ml castor oil p.o.).
iii)	EEMS1 (100 mg/kg with 0.5ml castor oil p.o.).
iv)	EEMS2 (200 mg/kg with 0.5ml castor oil p.o.).
v)	AEMS1 (100 mg/kg with 0.5ml castor oil p.o.).
vi)	AEMS2 (200 mg/kg with 0.5ml castor oil p.o.).

Different groups had given different treatment. After 30 minutes each animals was given 0.5 ml castor oil and placed in an individual perforated cage over a clean transparent filter paper. Then diarrhoeal episodes were observed for a periods of 4 hrs, during that period the time of onset diarrhoea, the total nos. of faecal output (frequency of defecation and weight of faeces excreted by the animals were recorded by using the formulae in terms of percentage of protection.

$$\% \text{ protection} = \frac{\text{Mean weight of stool of Control animals} - \text{Mean weight of stool of drug / extract treated animals}}{\text{Mean weight of stool of control animals.}} \times 100$$

Antimicrobial Susceptibility

Culture media

Nutrient agar was used for bacteria and Potato Dextrose Agar for fungi.

Standard drugs used for antimicrobial assay

Ciprofloxacin and Fluconazole were used as reference antibiotics against bacteria and fungi respectively.

Preparation of inocula

For the preparation of the inoculate 24h culture was emulsified in 3 ml sterile saline following the McFarland turbidity to obtain a concentration of 10^8cells/ml. The suspension was standardized by adjusting the optical density to 0.1 at 600 nm (ELICO SL-244 spectrophotometer). One hundred micro litres (100 µl) of cell suspension with approximately 10^6-10^8 bacteria per millilitre was placed in petridishes and dispersed over agar.

Agar well diffusion method

Antimicrobial activities of the crude extracts were first screened for their zone of inhibition by the agar well-diffusion method. Briefly, crude extracts were prepared concentration of 50 mg/ml and 100 mg/ml with dimethyl sulphoxide (DMSO) as solvent. The Mueller Hinton Agar (MHA) medium (Hi Media) was prepared and sterilised at 121°C 15 lb/sq for 20 min the autoclave. Thirty millilitres of this sterilised agar medium (MHA) were poured into each 9 cm sterile petridishes under aseptic conditions and allowed to settle. In the following, a well was made in the plates with the help of a sterile stainless steel-borer (6 mm diameter) two holes per plates were made into the set agar containing the bacterial culture. Each well 100 µl of the plant extracts at the various concentration. For each bacterial strain controls were maintained where pure solvents, instead of extract as negative control. Ethanol and Aqueous extracts (50 mg/ml and 100 mg/ml) and reference drug (Ciprofloxacin 100µg/ml) were allowed to diffuse for 1 h into the plates and then incubated at 37°C for 18h in inverted position. The results were recorded by measuring the zone of growth inhibition in mm surrounding the wells. Each assay was performed in triplicates and repeated twice [16].Both the fungal species was cultured in Potato Dextrose broth for 48h at 27°C and Savoured Dextrose Agar (SDA) was employed for the agar well diffusion experiments. Fungal suspensions were adjusted to10^7cells/ml as explained above. The zone of Inhibition was determined after incubation for 48h at 27°C.Here test drug ethanol and aqueous extracts (50 mg/ml) and (100 mg/ml) and standard drug fluconazole (100 µg/ml) were used respectively. All tests were performed in triplicates and repeated twice.

Minimal inhibitory concentration (MIC)

It is defined as the lowest concentration able to inhibit any visible bacterial growth on the culture plates. Sensitivity of the microorganisms of both ethanol and aqueous extracts of *M. scandens* can be measured by using tube dilution method where it can show the bactericidal or bacteriostatic. Each tube contained an inoculums density of $5x10^5$ CFU/mL of each of the test organisms. All organisms were grown in Muller Hinton broth. Then the suspension of all the four cultures was added into tubes containing diluted sample of *M. scandens* extracts 100-0.2µg/mL. The dilution of the samples was done with Mueller Hinton broth. Finally, the tubes containing diluted sample of *M. scandens* and bacteria was then incubated overnight at 37°C with constant shaking on the shaker. The growth of the microorganisms was determined by turbidity. Clear tubes indicated absence of bacterial growth. For every experiment, a sterility check (ethanol, medium) negative control (ethanol, medium, inoculums) and different standard antibiotics individually were included. The MIC of the samples was the lowest concentration in the medium that completely inhibited the visible growth. The solvent value was deducted accordingly to get the final results of activity [17-18].

Minimum Bactericidal concentration (MBC) and Minimum Fungicidal Concentration (MFC) assessment

The minimal bactericidal concentration (MBC) was determined by using the method of Vila *et al*.[19].To determine the MBC and minimal fungicidal concentration (MFC) of the plant extracts against the microorganisms, the plates of the MIC that showed no growth of the microbes were sub-cultured by striping using wire loop on sterile Muller Hinton agar plates. The plates were incubated at 37°C for 18-24 h and at

25°C for 48 h respectively for bacteria and fungi. The MBC and MFC were taken as the lowest concentration of the extract that exhibited not microbial growth on the agar plates [20].

Evaluation of bactericidal and bacteriostatic capacity

The action of an antibacterial on the bacterial strains can be characterized at two parameters as MIC and MBC. Accordingly to the ratio MBC/MIC, we can apperceive antibacterial activity. If the ratio MBC/MIC=1 or 2, effect is bactericidal but if the ratio MBC/MIC=4 or 16, effect is bacteriostatic [21].

RESULTS

Phytochemical Evaluation

The results of the phytochemical evaluation have showed the presence of flavonoids, alkaloids, saponins, steroids, glycosides, tannins and phenolic compounds Table 1

Acute Oral Toxicity Study

From this study, it was found that both the extracts of *M.scandens* were safe at 2000 mg/kg with no mortality.($1/20^{th}$ and $1/10^{th}$) of this dose i.s 100 mg/kg and 200 mg/kg were used in the antidiarrhoeal activity.

Table-1: Phytochemical screening of ethanol and aqueous extracts of *M. scandens*

Serial No.	Phytoconstituents	Ethanol Extract	Aqueous Extract
1.	Flavonoids	+	+
2.	Alkaloids	+	-
3.	Saponins	-	+
4.	Steroids	+	-
5.	Glycosides	+	-
6.	Tannins and Phenolic compound	+	+

Anti diarrhoeal activity

The anti diarrhoeal activity result observed that the aerial part of the ethanol and aqueous extracts of *M.scandens* showed significant anti diarrhoeal activity in dose dependent manner when compared to control which is evident by decrease in numbers and weight of the faecal matter. The average weight of the wet faeces of ethanol extract group at a dose of 100 mg/kg and 200 mg/kg was found 0.224±0.042 and 0.179±0.023 respectively. The average weight of the wet faeces of aqueous extract group at a dose of 100 mg/kg and 200 mg/kg was found 0.283±0.029 and 0.198±0.035respectively.For standard (loperamide) it was found to be 0.136±0.025.Hence it was found that the ethanol extract was more potent than aqueous extract. The results were shown in Table 2 and graphically represented (Figure 1,2 and 3).

Table-2: Effect of Ethanol and Aqueous extract of *aerial parts of M.scandens* on castor oil induced diarrhoea

Sl. No.	Treatment	Dose (mg/kg)	Avg. onset time of diarrhoea in min (mean ±SEM)	Avg. total no. of faeces (mean±SEM)	Avg. of No. of wet faeces (mean±SEM)	Avg. wt. of wet faeces (mg) (mean ±SEM)
1	Control	Distilled water	70.176 ±12.59	9.157± 0.735	6.833±0.687	0.357±0.042
2	Standard	Loperamide (3mg/kg)	200.83 ±21.615**	4.333 ±0.745**	3.023 ±0.575**	0.136 ±0.025***
3	EEMS1	100 mg/kg	145.636±25.897	6.567 ± 0.957	4.676± 1.015	0.224 ±0.042
4	EEMS2	200 mg/kg	185.83±23.653*	5.336 ±0.745*	3.351 ±0.512*	0.179±0.023**
5	AEMS1	100 mg/kg	131.17±23.769	7.653 ±0.942	5.167 ±0.645	0.283 ±0.029*
6	AEMS2	200 mg/kg	168.76 ±26.960*	6.321 ±1.022*	4.83 ± 0.984	0.198±0.035**

Values are expressed as mean ±SEM; n = 6 ; *P <0.05, **P<0.01 and ***P<0.001 when compared to control.

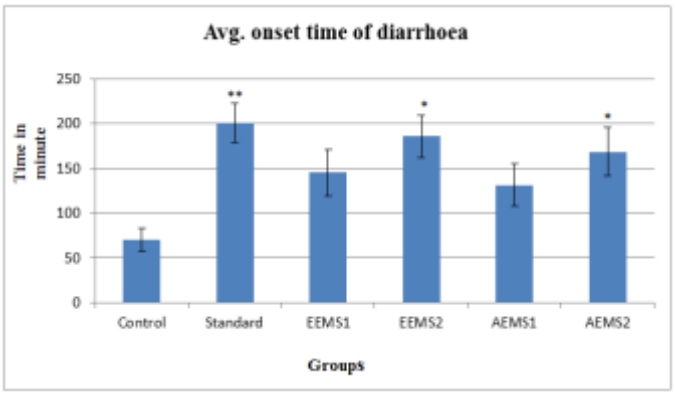

Fig-1: Average onset time of diarrhoea of different groups in castor oil induced diarrhoea

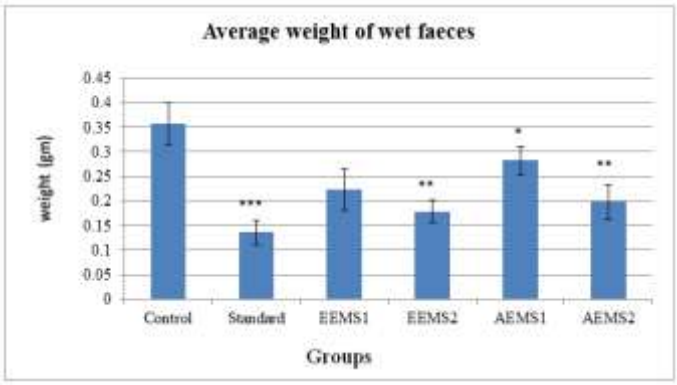

Fig-2: Average weight of wet faeces of different groups in castor oil induced diarrhoea

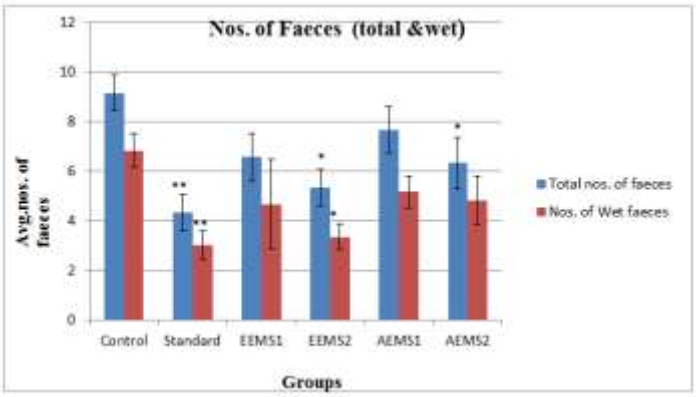

Fig-3: Number of faeces (total &wet) of different groups in castor oil induced diarrhoea

Table-3: Antimicrobial activity (Zone of inhibition, mm) of both Ethanol and Aqueous aerial extracts of *M.scandens against* clinical pathogens in Agar well diffusion assay

	Groups	Antibacterial activity (gram positive)				Antibacterial activity (gram negative)				Antifungal activity			
		Zone of inhibition (mm)											
		***B.Subtilis* MTCC441**		***S.aureus* MTCC29737**		***E.coli* MTCC1652**		***S.typhi* MTCC 733**		***C.albicans* MTCC227**		***A.niger* MTCC282**	
		50 mg/ml	100 mg/ml	50 mg/ml	100 mg/ml	50 mg/ml	100 mg/ml	50 mg/ml	100 mg/ml	50 mg/ml	100 mg/ml	50 mg/ml	100 mg/ml
1.	EEMS	13.3± 0.55	18.7 ± 0.18	17.5 ± 0.83	23.6 ± 0.33	19.2 ± 0.81	24.5 ± 0.93	13.4 ± 0.38	16.9 ± 0.43	23.7 ± 0.53	29.6 ± 0.81	19.5 ± 0.39	24.9 ± 0.33
2.	AQMS	10.3 ± 0.54	14.9 ± 0.32	13.4 ± 0.93	16.6 ± 0.81	12.5 ± 0.83	16.9 ± 0.13	10.9 ± 0.39	14.5 ± 0.73	17.5 ± 0.23	21.4 ± 0.63	16.8 ± 0.23	21.5 ± 0.53
3.	Control	-	-	-	-	-	-	-	-	-	-	-	-
4.	Ciprofloxacin 100 µg/ml	23±0.51		25.6±0.25		27.2±0.62		28.4±0.56					
5.	Fluconazole 100 µg/ml									34±0.35		31±0.55	

Values are expressed mean ± SEM, EEMS- Ethanol extract of *Mikania scandens*, AQMS- Aqueous extract of *Mikania scandens* .Standard antibacterial- Ciprofloxacin **(**100µg/ml**),** Antifungal- Fluconazole **(**100µg/ml**),** Control- DMSO,

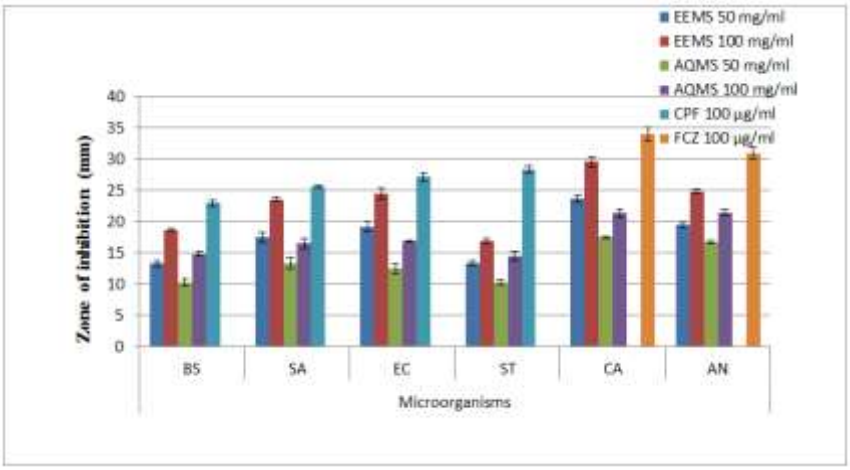

Fig-4: Effect of *M. scandens* Ethanol and aqueous extract on Zone of inhibition of different pathogenic microorganisms

BS- Bacillus subtilis, SA-Staphylococcus aureus, EC-Escheria coli, ST-Salmonella typhi, CA-Candida albicans,AN-Asperigillus niger.CPF-Ciprofloxacin,FCZ- Fluconazole. EEMS- Ethanol extract of *Mikania scandens*, AQMS- Aqueous extract of *Mikania scandens*

Antimicrobial activity

In this study, four bacteria strains (Gram positive and Gram negative bacteria) were used. The antimicrobial assays were performed by agar well diffusion method and tube dilution method so that they could be qualified and quantified by inhibition zone diameter, MIC, MBC and MFC also. We observed that the susceptibility of the bacteria to the ethanol extract on the basis of inhibition zone diameters possess potent antimicrobials against all the tested microorganisms (*B.subtilis, S. aureus, E.coli, S. typhi)* in dose dependent manner. The highest inhibition activities were observed the ethanol extracts of *M. scandens* against the *S.aureus* (gram positive) were 17.5±0.83 and 23±0.33 and 19.2±0.81 and 24.5±0.93 against *E.coli* (gram negative) at the same concentration (Table 3). Ciprofloxacin at a concentration (100 µg/ml) fully inhibited the growth of all the bacterial stains.

The antifungal activity was also studied for both extracts using two fungal strains namely *C.albicans and A.niger.*In the present study maximum antifungal activity was observed 23.7±0.53 and 29.6±0.81 for the ethanol extract against *C.albicans* at the similar concentration.

Table-4: MIC and MBC determination, bactericidal (+) and bactriostatic (-) effect of the ethanol and aqueous extracts of *M.scandens*

	Ethanol Extract				Aqueous Extract			
Microorganisms	MIC (µg/ml)	MBC (µg/ml)	MBC/MIC	Effect	MIC (µg/ml)	MBC (µg/ml)	MBC/MIC	Effect
Staphylococcus aureus	128	256	2	+	128	512	4	-
Bacillus subtilis	256	1024	4	-	1024	NA	NA	-
Escherichia Coli	512	1024	2	+	256	512	2	+
Salmonella typhi	128	512	4	-	128	512	4	-

NA-No activity

Minimal inhibitory concentration (MIC)

The minimal inhibitory concentration (MIC) obtained with both ethanol and aqueous extracts of *M.scandens* were 1-1024µg/ml. Here the results were varied according to the microorganisms. (Table 4)

Minimal bactericidal concentration (MBC) and minimal fungicidal concentration (MFC)

The minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) values were ranges from 1 -1024 µg/ml. The bactericidal and bacteriostatic effect was determined using the ratio MBC/MIC and MFC/MIC. (Table 5)

Table-5: MFC determination, bactericidal (+) and bacteriostatic (-) effect of the ethanol and aqueous extracts of *M. scandens*

	Ethanol Extract				Aqueous Extract			
Microorganisms	MIC (µg/ml)	MFC (µg/ml)	MFC/MIC	Effect	MIC (µg/ml)	MFC (µg/ml)	MFC/MIC	Effect
Candida albicans	256	512	2	+	128	512	<2	-
Asperigillus niger	128	1024	4	-	256	NA	NA	-

DISCUSSION

Humans commonly using plant(s) or plant derived preparations regard them to be effective against diarrheal disorders without any scientific basis to explain the action of such plants. Some plants have been evaluated for their anti-diarrheal properties [22-23]. The aim of this study was to experimentally evaluate the acclaimed use of *M. scandens* aerial parts, which are considered to consult preventative in diarrhoea in Ethiopian traditional medicine. Various studies have accepted the use of antidiarrheal medicinal plants by investigating the biological activity of the extracts of such plants, which have delay intestinal transit, reduce gut motility, stimulate water absorption, diminish the intraluminal fluid accumulation[24].This experimental model was therefore employed to validate antidiarrheal efficacy of *M. scandens* extracts in the present study.

Several mechanism have been early recommended to explain the diarrhoeal effect of castor oil including inhibition of intestinal Na^+, K^+ ATPase activity to decrease normal fluid absorption, activation of adenylate cyclase or mucosal cAMP mediated active secretion, stimulation of Prostaglandin formation platelet activating factor and nitric oxide has been declared to contribute to the diarrheal effect of castor oil However it is well evident that castor oil produces diarrhoea due to its active metabolite, ricinolic acid [25]which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. Its action also stimulates the release of endogenous prostaglandin [26]. Castor oil reported to induce diarrhoea by increasing the volume of intestinal contents by arresting the re-absorption of water. The liberation of ricinoleic acid results in irritation and inflammation of intestinal mucosa leading to release of prostaglandin [27]. In this study the ethanol extract of *M. scandens* aerial parts exhibited a significant dose dependent anti diarrhoeal activity comparable with the standard drug loperamide.

The ethanol extract of the aerial parts of the *M.scandens* showed broad spectrum of antimicrobial activity. It was perceived in the present study that ethanol extract inhibited the growth of all pathogenic microorganisms tested moderately. Among these bacteria, *E. coli, S. typhi, B. subtilis, S.aureus* can cause dangerous such as diarrhea, dysentery, typhoid fever and other intestinal diseases to the human beings. However *M. scandens* extract was found to be active against the above Gram negative bacteria. The plants extract exhibit appreciable activity against Gram positive bacterial strains.

Preliminary phytochemical screening of *M. scandens* revealed that the presence of a number of a bioactive constituents such as flavonoids, tannins, saponins, alkaloids. The antimicrobial activity could be due to the presence of these phytoconstituents. Flavonoids and tannins in commonly have been reported to have antidiarrheal activity through inhibition of intestinal motility, antimicrobial action and ant secretory effects [28].The bactericidal activity could be described to the presence of polyphenols compounds. Polyphenols, such as flavonoids and tannins are of important antibacterial activity [29,30]

CONCLUSION

The results of this investigation showed that ethanol extracts were mostly prominent effective antimicrobial agents against specified microorganisms incriminated in gastrointestinal infectious diseases such as diarrhoea and dysentery. *M.scandens* aerial extracts has shown the best bactericidal, fungicidal and anti-diarrheal activity. The results produced the rationale for the use of this extracts by traditional healers as antidiarrheal drugs.

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