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PHYTOCHEMICAL AND ANTIBACTERIAL INVESTIGATION OF LEAVES OF ACACIA *NILOTICA* AGAINST ANTIBIOTIC ASSOCIATED DIARRHOEA (AAD) PATHOGENS

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ABSTRACT

Due to the presence of various phytochemicals in Medicinal plants, they are used for curing of various human diseases and process of healing. Plants are very useful and utilized as medicine due to their medicinal properties. Screening of plants for biologically active compounds against human pathogens is a renewed interested research field.The genus *Acacia* belongs to family Mimosaceae. Trees, *Acacia nilotica* (linn), Wild ex Del is known in India as babul, kikar, Babur (Hindi) is a very large genus containing shrubs and climbers. *A. nilotica* has played an important role in the traditional medicine. Thus, the modern pharmacological and clinical investigation of *A. nilotica* leaf is a valuable herbal therapy that has an antioxidant, antimicrobial, anti-inflammatory, anti-viral and antiulcer properties. In the present study, principal phytoconstituents of *Acacia nilotica* were identified in order to relate their presence with bioactivities of the plants. This study evaluated the proximate composition and phytochemicals present in the leaves. Phytochemical screening of the different extracts of the plant was performed using standard methods and resulted in the detection of the presence of alkaloids, flavonoids, glycosides, saponins, phenols, tannins and terpenoids in methanolic and aqueous extract. Antibacterial activities were tested against bacterial strains shows good results. When compared to other extracts, it was revealed that the methanolic extract with a concentration of 50 mg/ml showed the highest zone of inhibition among all the microorganisms. A large number of the medicinal plants are used extensively by the tribal people worldwide as these plants claims to possess the antibacterial properties in the traditional system and it is now believed that nature has given the cure of every disease in one way or another.

KEYWORDS

Acacia Nilotica, Antibiotic Associated Diarrhea, Phytochemical screening and Antibacterial assay.

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INTRODUCTION

Millions of friendly bacteria live in our intestines. They are essential to digestion. But diarrhoea can throw the microbes in your gut off balance, and vice versa. The discovery of antibiotics in the early twentieth century provided an increasingly important tool to combat bacterial diseases. Antibiotics kill the bad germs in your body that

make you sick, but they also kill the good bacteria. This can disrupt the normal balance in your intestines, leading to diarrhoea. As antibiotics are increasingly used and misused, the bacterial strains become resistant to antibiotics rapidly. Hence, there is a pressing need to develop new and innovative potent sources which can be used as antimicrobial agents[1]. Among the potential sources of new agents, plants can be used as one source. Because, they contain many bioactive compounds that can be of interest in therapeutic. More over there has been an increase demand for the herbal products of Ayurveda in all over the world because of fact that the allopathic drugs have a side effect .*Acacia nilotica L.* commonly known as 'Babul' or 'Kikar' belongings to the family Mimosaceae is a medium sized tree. *Acacia* is the most significant genus of family Leguminosae. It tolerates extremes of temperature and moisture. It is suited for planting on marginal lands and can survive both drought and flooded conditions. It has an inspiring range of medicinal uses with potential anti-oxidant activity. The plant is considered to be antispasmodic and antidysenteric. The leaves are used for the treatment of diarrhoea[2]. The digestive tract is a complex ecosystem that's home to millions of microorganisms (intestinal flora), including hundreds of species of bacteria. Many of these bacteria are called "good" bacteria as they helps in performing essential functions. On the contrary some of the bacteria called "bad" bacteria that normally inhabit your intestinal tract are potentially dangerous. These harmful bacteria are usually kept in check by beneficial bacteria unless the delicate balance between the two is disturbed by illness, medications or other factors. One of the major side effect of using Antibiotics is that they are a destructive agent to intestinal flora because they destroy beneficial good bacteria along with harmful bad ones. Sometimes, without enough "good" microorganisms, "bad" bacteria that are resistant to the antibiotic you received grow out of control, producing toxins that can damage the bowel wall and trigger inflammation. In the first case reduce metabolism of fermentable carbohydrate lead to reduce short chain fatty acid and increase non absorbable carbohydrates in the gut, the protective barrier provided by the normal intestinal micro flora. Is disrupted and this lead to reduction in the ability of the gut to resist colonization by pathogen. As a result opportunistic growth of pathogen occurs for example *C. difficile.* The study is planned in a way to establish the bio efficacy potential of extracts of different parts of *Acacia nilotica* against AAD pathogens[3]. Hence this study aims to prevent this diarrhea by interrupting either of the potential mechanisms:-

- By maintaining the flora of the gut and ongoing carbohydrate fermentation
- By competitively inhibiting the growth of pathogens.

MATERIAL AND METHODS

Collection of plant materials

The leaves of *Acacia Nilotica* were collected from University of Rajasthan and identified and authenticated from the herbarium, Dept. of Botany, UOR.

Preparation of plant extracts

The leaves were washed with clean sterile distilled water and shade dried to reduce water content. Then the dried plant leaves were crushed into fine powder using mortar and pestle. The different solvent extracts (methanol, aqueous, chloroform and petroleum ether) from leaves of *Acacia nilotica* were prepared in accordance with the standard procedures through soxhlet extraction.

Collection of isolates

The test organism was procured from the repository of Dr. B. Lal Institute of Biotechnology.

Antibacterial assay of the extract against the isolate

Comparative analysis of Antibacterial activity of different extracts of *Acacia nilotica* against the test isolates (*Staphylococcus aureus, Escherichia coli, Salmonella typhi, Shigelladysenteriae*) were performed in accordance with standard procedures.Streptomycin (0.1g /ml) was used as positive control and respective solvents (methanol, aqueous, chloroform, acetone and petroleum ether

solvent, 50 µl) was used as negative control. The antibacterial assay plates were incubated at 37˚C for 24 hrs. After incubation, antibacterial activity was determined by measuring the zone of inhibition in millimetre scale against the studied bacteria.

Phytochemical screening of the extracts for the presence of bioactive component

The phytochemical screening of the *Acacia nilotica* plant was carried out in accordance with standard protocols for the presence of various bioactive components (qualitative) like tannins, flavonoids, saponins, glycosides, terpenoids : Tannin with (FeCl3), carbohydrate with (alcoholic α-naphthol solution, Benedict's reagent), Alkaloids with (Mayer and Dragendoff's reagents), Saponins (foaming test), Flavonoids (chip of magnesium and HCl), Glycosides (NaCl, and Felhing's solutions A and B), Phenols -(FeCl3), Terpenoids- (Salkowshi test)

RESULTS AND DISCUSSION

Antibacterial activity of *Acacia Nilotica*

The antimicrobial potential of five extracts was screened against *Staphylococcus aureus, Salmonella, Shigella* and *E coli* the results of antibacterial activity of all extracts i.e. methanolic, aqueous, acetone, chloroform and petroleum ether against *Staphylococcus aureus* are shown in Table No.1. The zone of inhibition shows a range from 20±1mm to 25.5±0.5 mm in methanolic extracts and from 14.66±0.57 mm to 17.6±0.57 mm in aqueous extracts with different concentrations of the extract respectively. On the other hand Table No.2. Revealed the results of antibacterial activity of all extracts against *Escherichia coli*. The zone of inhibition shows a range from 20±1mm to 28±1 mm in methanolic extracts and from 10.33±0.57 mm to 20.26±0.25mm in aqueous extracts with different concentrations of the extract respectively. The zone of inhibition shows a range from 26.83±0.28mm to 26±0.5mm in methanolic extracts against *Salmonella typhi* and from 16.5±0.5mm to 23±0.5 mm in aqueous extracts with different concentrations of the extract respectively against *Salmonella typhi* (Table No.3). The results of antibacterial activity of all extracts against *Shigelladysenteriae* are shown in Table No.4. The zone of inhibition shows a range from 28±1 mm to 32.5±0.5 mm in methanolic extracts and from 32.5±0.5mm to 26.83±0.28 mm in aqueous extracts with different concentrations of the extract respectively. However no significant zone of inhibition was recorded for acetone, chloroform and petroleum ether extracts against all the isolates.

Phytochemical analysis of *Acacia Nilotica*

Phytochemical screening for the presence/ absence of bioactive components of the leaves of *A. nilotica* (methanolic and aqueous extract) revealed that the plant contains terpenoids, alkaloids, tannins, phenols, glycosides, saponins and flavonoids. The phytochemical analysis of the acetone extract of *Acacia nilotica* leaves revealed that tannins, saponins, flavonoids, alkaloids, phenol, glycosides are present while terpenoids were absent. The phytochemical analysis of the chloroform extract of *Acacia nilotica* leaves revealed that saponins, alkaloids, phenol, glycosides are present while terpenoids, flavonoids and tannins were absent. The phytochemical analysis of the petroleum ether extract of *Acacia nilotica* leaves revealed that tannins, saponins, flavonoids, glycosides are present while alkaloids, phenols and terpenoids were absent. (Table No.5).

Table No.1: Results of antibacterial activities of *Acacia nilotica* leaf extracts against *S. Aureus*

S.No	Concentration (mg/ml)	Aqueous Extract		Methanolic Extract		Chloroform Extract		Acetone Extract		Petroleum Ether Extract	
1	25	IZ	14.66±0.57mm	IZ	20±1mm	IZ	NS	IZ		IZ	NS
		AI	0.54	AI	0.86	AI		AI		AI	
2	35	IZ	16±1mm	IZ	22±0mm	IZ	NS	IZ		IZ	NS
		AI	0.59	AI	0.95	AI		AI		AI	
3	50	IZ	17.6±0.57mm	IZ	25.5±0.5mm	IZ	NS	IZ		IZ	NS
		AI	0.65	AI	1.10	AI		AI		AI	

IZ – zone of inhibition, AI – Activity index, NS - non significant, Values are mean inhibition zone (mm) ± S.D of three replicates

Table No.2: Results of antibacterial activities of *Acacia nilotica leaf* extracts against *E. Coli*

S.No	Concentration (mg/ml)	Aqueous Extract		Methanolic Extract		Chloroform Extract		Acetone Extract		Petroleum Ether Extract	
1	25	IZ	10.33±0.57mm	IZ	20±1	IZ	NS	IZ		IZ	NS
		AI	0.31	AI	0.62	AI		AI		AI	
2	35	IZ	11.23±0.25mm	IZ	23.5±0.5	IZ	NS	IZ		IZ	NS
		AI	0.34	AI	0.73	AI		AI		AI	
3	50	IZ	20.26±0.25mm	IZ	28±1	IZ	NS	IZ		IZ	NS
		AI	0.61	AI	0.63	AI		AI		AI	

IZ – zone of inhibition, AI – Activity index, NS - non significant, Values are mean inhibition zone (mm) ± S.D of three replicate

Table No.3: Results of antibacterial activities of *Acacia nilotica* leaf extracts against *Salmonella*

S.No	Concentration (mg/ml)	Aqueous Extract		Methanolic Extract		Chloroform Extract		Acetone Extract		Petroleum Ether Extract	
1	25	IZ	16.5±0.5mm	IZ	26.83±0.28mm	IZ	NS	IZ		IZ	NS
		AI	0.5	AI	0.86	AI		AI		AI	
2	35	IZ	20±1mm	IZ	30.1±0.1mm	IZ	NS	IZ		IZ	NS
		AI	0.60	AI	0.97	AI		AI		AI	
3	50	IZ	23±0.5mm	IZ	26±0.5mm	IZ	NS	IZ		IZ	NS
		AI	0.69	AI	0.83	AI		AI		AI	

IZ – zone of inhibition, AI - Activity index, NS - non significant, Values are mean inhibition zone (mm) ± S.D of three replicates

Table No.4: Results of antibacterial activities of *Acacia nilotica* leaf extracts against *Shigella*

S.No	Concentration (mg/ml)	Aqueous Extract		Methanolic Extract		Chloroform Extract		Acetone Extract		Petroleum Ether Extract	
1	25	IZ	25.1±0.28mm	IZ	28±1mm	IZ	NS	IZ		IZ	NS
		AI	0.83	AI	0.84	AI		AI		AI	
2	35	IZ	21±1mm	IZ	30±0mm	IZ	NS	IZ		IZ	NS
		AI	0.7	AI	0.90	AI		AI		AI	
3	50	IZ	26.83±0.28mm	IZ	32.5±0.5mm	IZ	NS	IZ		IZ	NS
		AI	0.89	AI	0.98	AI		AI		AI	

IZ – zone of inhibition, AI - Activity index, NS - non significant, Values are mean inhibition zone (mm) ± S.D of three replicates

Table No.5: Results of phytochemical screening of *Acacia nilotica* different extracts

S.No	Active principle	Methanolic Extract	Aqueous Extract	Chloroform Extract	Petroleum Ether Extract	Acetone Extract
1	Alkaloids	+	+	+	-	+
2	Terpenoids	+	+	-	-	-
3	Flavanoids	+	+	-	+	+
4	Saponins	+	+	+	+	+
5	Tannins	+	+	-	+	+
6	Phenol	+	+	+	-	+
7	Glycoside	+	+	+	+	+

+ = presence of phytoconstituents, - = absence of phytoconstituents

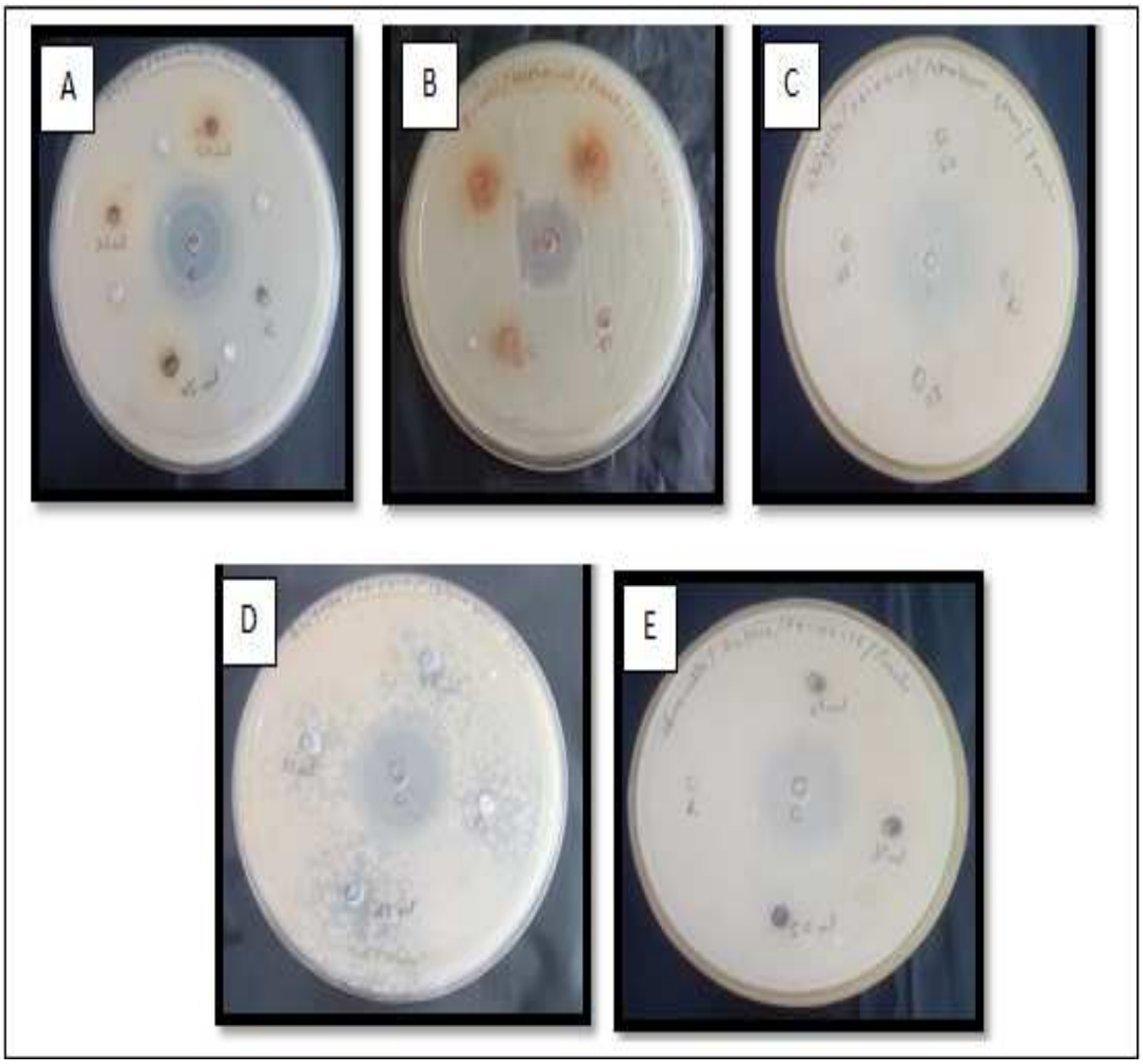

Figure No.1: Inhibition Zone of different extracts against *E.coli.* A-aqueous, B-methanol, C-petroleum ether, D-chloroform, E- acetone

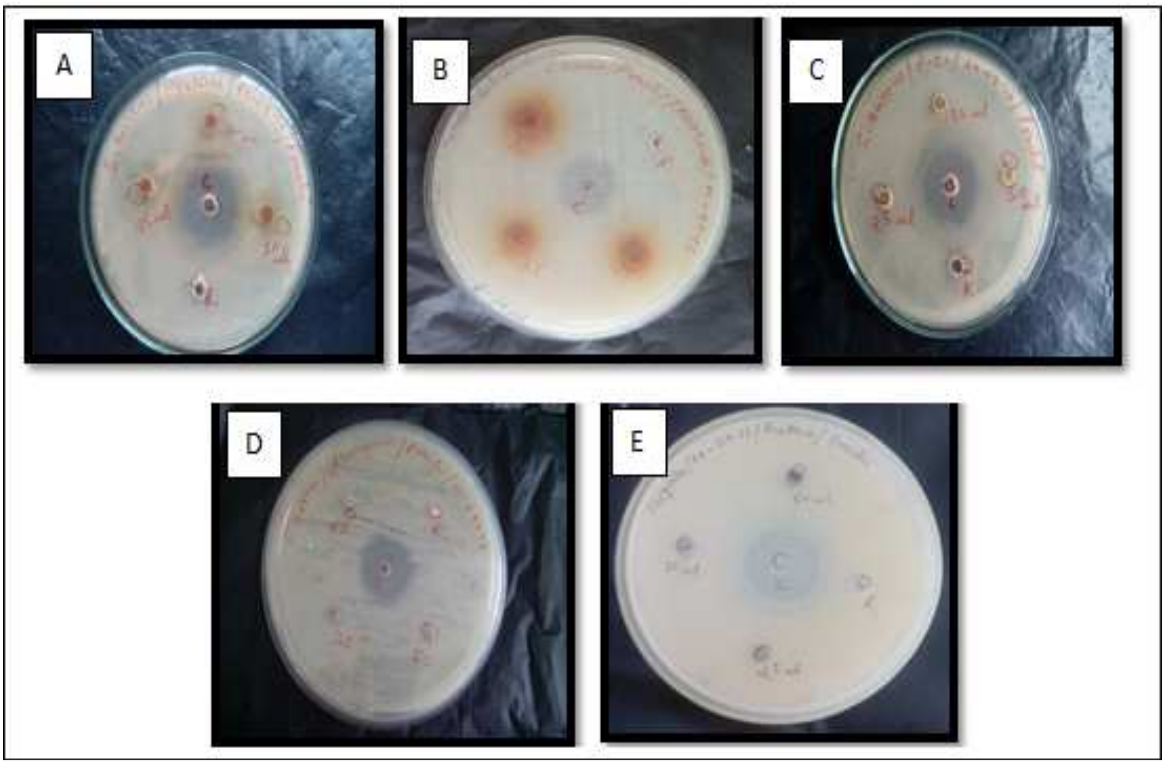

Figure No.2: Inhibition Zone of different extracts against *S. aureus*. A- aqueous, B- methanol, C-petroleum ether, D-chlofororm, E-Acetone

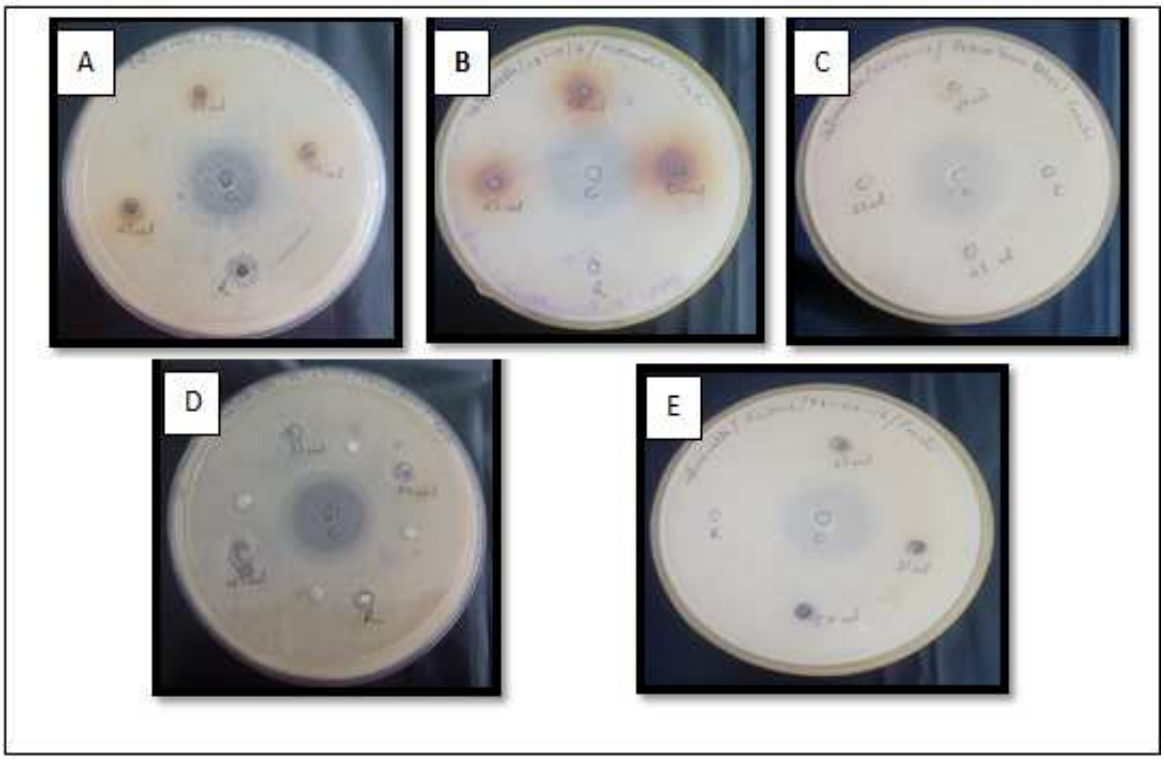

Figure No.3: Inhibition Zone of different extracts against *S. typhi*. A-aqueous, B-methanol, C-petroleum ether, D-chloroform, E-acetone

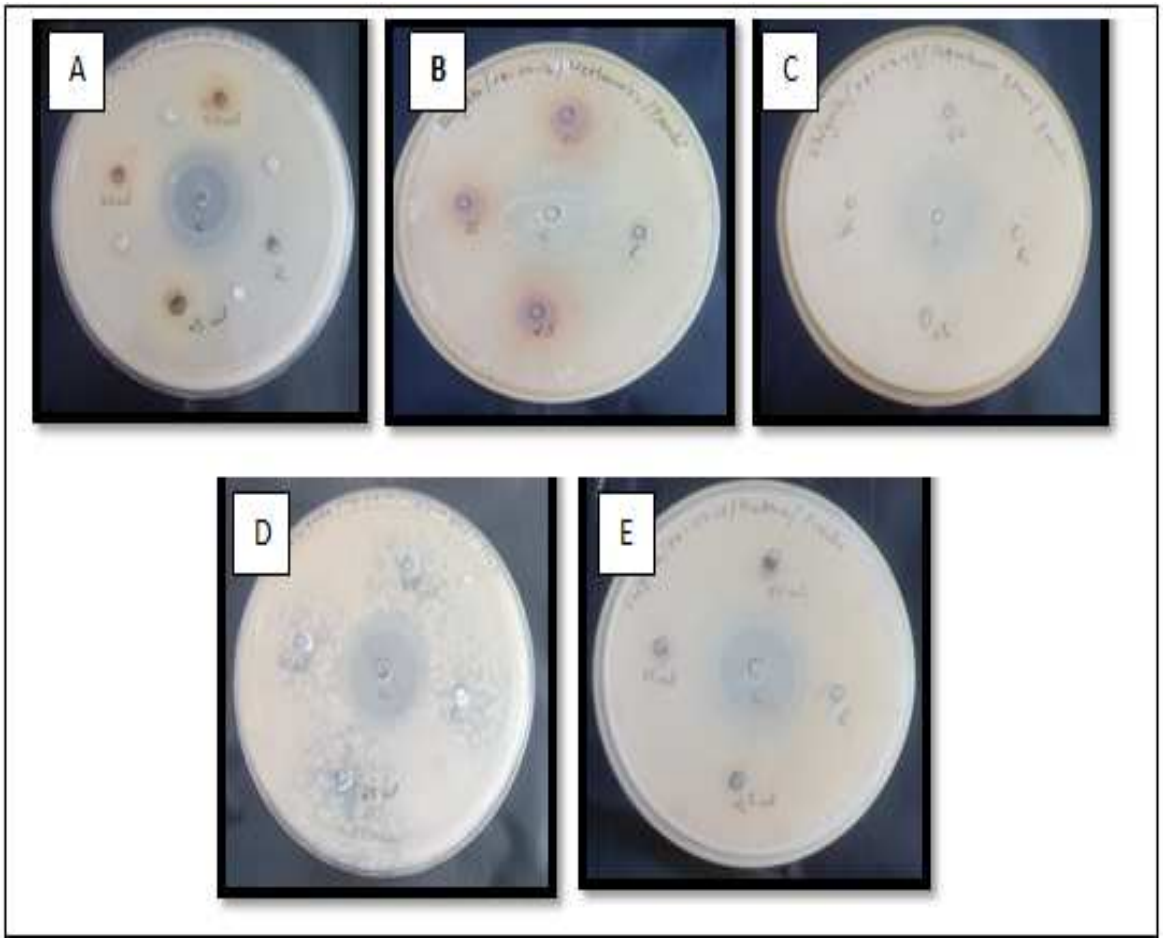

Figure No.4: Inhibition Zone of different extracts against *S. dysenteriae*. A-aqueous, B-methanol, C-petroleum ether, D-chloroform, E-acetone

CONCLUSION

The active principles identified in the present investigation clearly indicate that the methanolic and aqueous extracts shows potent antibacterial activity in inhibition of *S. aureus and E coli* and thus these extracts of *Acacia nilotica* could be used as a potent source to combat Antibiotic Associated Diarrhea. Furthermore, high throughput screening methods are required for a comparative study on the quantitative analysis of the phytochemical properties of different extracts and hence determining their medicinal properties targeted against AAD pathogens. Further research is needed toward further isolation and identification of active principles present in the column fractions which could possibly be exploited for pharmaceutical use.

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CONFLICT OF INTEREST

There is no conflict of interest declared by the author.

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