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Bacterial inhibition effects and phytochemical analysis of Anacardium occidentale against common diarrhoeal pathogens

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ABSTRACT *Diarrheal samples were collected from the patients of Government hospital, Srirangam and the causative pathogens were isolated and identified as E. coli, Salmonella sp. & Klebsiella. Leaves of Anacardium occidentale were collected and microbial inhibition activity of their aqueous and ethanol extracts were screened against the isolated diarrheal pathogens. The results found that ethanol extracts showed maximum inhibition effect and contains major phytochemical compounds.*

KEYWORDS : Diarrheal pathogens, antimicrobial activity, phytochemical compounds

Introduction

Natural products based compounds have an immerse impact of modern medicine about 40% of prescription drugs are based on them. Medicinal plants are potential source of drugs playing an important role in the world's economy. Over 60% of world human population and 80% in the developing countries depends directly on plants for the medicinal purposes (Abeysinghe, 2010). Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs (Varaprasad, 2009).

The antimicrobial properties of various medicinal plants have been exploited over the years in the treatment of diseases all over the world. Plant species have been used in the formulation of various proprietary medicines and it is therefore important to determine the active chemical components of such plants in order to develop more effective drugs. Plant products still remain the principal source of pharmaceutical drugs and agents used in traditional medicine. The effects of plant extracts on bacteria have been studied by a large number of researches in different parts of the world (Priyatharsini et al, 2015).

Anacardium occidentale is a tree belonging to family Anacardiaceae and it contains cashew nutshell liquid (CNSL), a by-product of processing cashew, is mostly composed of anacardic acids. These acids have been used effectively against tooth abscesses due to their lethality to gram-positive bacteria. The bark is scraped and soaked overnight or boiled as an antidiarrheal and the seeds are ground up into powders used for antivenom for snake bites. The nut oil is used topically as an antifungal and for healing cracked heels (Ateb & Erdourul, 2003). Due to the fact that the plant *Anacardium occidentale* is very useful, as found by above mentioned reports and there is a need to find out more about the potentiality of this plant as an antimicrobial agent. Therefore, the present study was designed to assess the potency of *Anacardium occidentale* against the diarrheal causing pathogenic bacteria.

Materials and methods

Collection of diarrheal samples and isolation of causative bacteria

Diarrheal samples were collected from Government hospital, Srirangam and the samples were placed in transport media containing phosphate buffer saline (PBS) for transportation. In continuation, the samples were serially diluted and inoculated into selective and differential media and incubated at 37ºC for 24 h. After incubation, the colonies were subjected for identification by adopting standard procedures indicated in the Bergey's Manual of determinative bacteriology (Priyatharsini et al, 2015).

Collection and organoleptic evaluation and extraction of plant materials

Leaves of *A. occidentale* were collected from Trichy, Tamil Nadu and it was ground to uniform coarse powder, after shadow dried. The plant materials were subjected to macroscopic studies which comprised of organoleptic characters of the drugs viz., colour, odour, appearance, taste, smell, texture, fracture, etc. About 200 gm of plant material powder was taken and extracted with six parts of water and boiled till the total content was reduced to one third and filtered. The filtrate obtained was evaporated to dryness, till it becomes paste form, this paste form of the extract was used for the study. The ethanol extracts were prepared by soaking 100 g each of the dry powdered plant material in one liter of ethanol at room temperature for 72 hours. The extracts were filtered through a Muslin cloth then using whattman filter paper No 42 (125mm). The extracts were concentrated using a rotary evaporator with water bath set at 60˚ C.

Microbial analysis of plant materials

Investigation of microbial quantity was carried out in the plant extracts followed by standard protocol. Total enumeration of aerobic, enteric bacteria and fungi were recorded by pour & spread plate method with nutrient agar, SS agar and Rose Bengal Chloromphenical agar respectively.

Bacterial inhibition assay

Inhibition activity of aqueous and ethanol extracts of *A. occidentale* were tested against the pathogens by disc diffusion method (Priyatharsini et al, 2015). The standard inoculums suspension was swabbed over the surface of the media using sterile cotton swab to ensure the confluent growth of the organism. The 5 mm diameter discs were prepared with Whatman No.1 paper. Different concentration (200,400,600,800 µg) of extract was impregnated on the filter paper discs. The discs were placed on the surface of the plate with sterile forceps and pressed gently to ensure contact with the inoculated agar surface. Standard antibiotic was used as positive reference to determine the sensitivity of the tested strains and ethanol and aqueous was used as negative control. Finally, the inoculated plates were incubated at 37^{0}C for 24 hrs and the inhibition zones were observed including the diameter of the disc. All the experiments were carried out in triplicate.

Determination of Minimum Inhibitory Concentration (MIC)

Stock concentration of various plant extract was prepared by making use of DMSO: Methanol, in the ratio of 1:1 which in turn was diluted with equal volume of phosphate buffered saline, pH 7. Muller Hinton Agar was prepared, sterilized and kept ready in molten condition. 20 ml of the molten media was taken and was mixed with known concentration of different extracts / fractions and were added in different tubes. This mixture was swirled carefully for complete mixing of extract and media and poured on to the plate. After getting solidified it was inoculated with the test organism and standard organism. The plates were incubated at 37º C for 24 hours. MIC was recorded based

on the growth of the organisms.

Qualitative analysis of phytochemical compounds

Alkaloids, steroids, terpenoids, flavanoids, tannins, flavones, cardiac glycoside, proteins, carbohydrate, ninhydrin, lignin and inulin were analysed by adapting the method proposed by (Dhayanithi et al, 2012).

Results and discussion

Diarrhoeal disease is responsible for one fourth of all death among children under 5years of age in developing countries. Bacterial etiologies associated with diarrhoea are *E. coli* (50%), *Salmonella* (20%), *Klebsiella* sp (20%) and *Shigella* sp (10%). Banaje *et al*., (2004) also showed *E. coli* 58%, *Shigella* 20% incidence and our results also in lined with their report. The present study also identified *E. coli*, *Salmonella* sp. & *Klebsiella* (Table-1 & 2).

Medicinal plants are the backbone of traditional system of medicines like Ayurveda and Siddha. India is blessed with diversity of plant species have good medicinal value. Good raw material provides better therapeutic values. Pharmaceuticals study will contribute in determining characteristic features of standard and raw materials. The medicinal plant contains active constituents that are used in the treatment of many human diseases. Many of the plant used in traditional medicine are readily available in rural areas at relatively cheaper than modern medicine (Sadiq et al, 2009). Thus it is important to charecterize different type of medicinal plants for their antioxidant and antimicrobial potential. The organoleptic evaluation and microbial quality of the plant materials were analysed and reported in table -3.

Phytochemical evaluation was performed for qualitative detection of various chemical constituents which aid in tracing the presence of active entity that elicit a major pharmacological response. Earlier researchers (Abulude et al, 2010; Ayepola & Ishola 2009; Chung et al, 1998) described that phenolic content like tannins, flavonoids found plants may be responsible for antibacterial activity. These compounds precipitate surface proteins of microorganism's therapy inhibit/arrest the growth of microorganisms. This result is similar to the present study which showed the presence of alkaloids, terpenoids, tannins, flavones, inulin, amino acids, ninhydrin in alcoholic extracts and alkaloids, terpenoids, tannins, lignins, flavones, inulin, amino acids, ninhydrin in aqueous extract respectively (Table-4). It has been stated that the mechanism of the antimicrobial activity of the plant extract involves the inhibition of various cellular processes, increase in plasma membrane permeability and impairment of energy or synthesis of structural components in microbial cells (Chung et al, 1998).

Phytochemical analysis of *Anacardium occidentale* L. Nuts: A variety of rich metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides and volatile oils are present in plants in general (Ayepola & Ishola, 2009). In the present study, ethanolic extract of *A. occidentale* L. shows the presence of various phytochemical compounds such as triterpenoids, phenolics and volatile oils, etc. Phytochemical analysis had revealed the presence of alkaloids, polyphenols, saponins etc. and based on the increased number of reports on blood glucose retardation associated with some saponins and alkaloids isolated from other medicinal plants, it is likely that the active principles could be present in one or the two families of the chemical substances (Muthuselvam, 2016; Ayepola & Ishola, (2009); Chung et al, 1998).

Disc diffusion method was adopted to screen antibacterial activities of different plant extracts. Aqueous and alcohol extracts were collected from the plant material and subjected to antimicrobial screening assay. Leaves of alcoholic extract of *Anacardium occidentale* showed best activity (19mm) against *Salmonella* sp. at 800µg/disc concentration. Aqueous extract produced 13mm against *E.coli* at 600µg/disc concentration (Figure-1). In addition that ethanolic and aqueous extracts produced MIC at 450µg/ml and 750µg/ml against *Klebsiella* sp (Table-5).

Different plants aqueous and alcoholic extracts were assessed for its antimicrobial potency in previous studies. Results revealed that the extracts produced at least 6mm of zone of inhibition against all the organisms tested at 200µg/disc concentration. Results of this study have shown that both aqueous and ethanolic extracts of *Anacardium occidentale* possess antibacterial activities. This observation may be due to the presence of bioactive substances in both the leaves extracts (Jothi et al, 2013). Our results were similar to that of the previous results (Ayepola & Ishola, 2009; Jothi et al, 2013; Muthuselvam, 2016) were ethanol extract was more efficacious than the aqueous extract. This observation in this study may be due to the ability of the active ingredient present in *Anacardium occidentale* to dissolve more in ethanol than in water.

Phenolic compounds present in the plant material may precipitate the protein available on the surface of the bacterium. But, flavanoids reduce the motility of the bacterium. Flavanoids & tannins may be major group of compounds that act as primary antioxidants or free radical & scavenges. The ratios of the components were estimated of the plant materials from different parts of the country. Based on the phytochemical ratio, the plant extracts will act potential role in the bacterial inhibition activity, antioxidant assay, etc. In conclusion, the outcome of this study has demonstrated the potential of bacterial inhibition of *Anacardium occidentale* leaves against diarrheal causing pathogenic bacteria.

Conclusion

The present work confirm the potential pattern of *Anacardium occidentale* against diarrheal casing bacteria with the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings.

Table-1: Identification features based on bacterial colonies on the selective media

S. No	Medium	*E. coli sp.*	Salmonella sp.	Enterobacter sp.
1.	Nutrient agar	White colour colonies	White colour colonies	White colour colonies
2.	Macconkey agar	Lactose ferment colonies	Non lactose ferment colonies	Red mucoid colonies
3.	Blood agar	Non-haemolytic	Haemolytic	Non-haemolytic
4.	EMB agar	Green matalic sheen colonies	Not performed	Not performed
5	SS agar	Pink colour	White mucoid colonies	NP

Table-2: Biochemical features and identification keys of pathogenic isolates

S.No	Test	*E. coli sp.*	*Salmonella sp.*	*Klebsiella sp*
1	Simple staining	Rod	Rod	Rod
2	Gram staining	-	-	-
3	Motility	Motile	Motile	Non motile
4	Indole	+	-	-
5	Methyl Red	+	+	-
6	Vogesproskaur	-	-	+
7	Citrate utilization	-	-	+
8	Urease	-	-	+
9	TSI	Acid/Acid	Acid/Alkaline	Acid/Acid
	H_2S	-	+	-
	Gas	+	+	+
10	Nitrate reduction	+	+	+
11	Catalase	+	+	+
12	Oxidase	+	+	+
13	Carbohydrate fermentation Test			
	Glucose	+	+	+
	Maltose	+	+	+
	Sucrose	+	+	+
14	Coagulase	+	+	+

Table - 3: Microbial limit assay of *Anacardium occidentale*

S. No	Description	Microbial counts (CFU/g)
1	Aerobic bacteria count	50×10^2
2	Fungal count	Nil
3	Enteric bacteria count	Nil
4	*E.coli sp.*	Nil
5	*Salmonella sp.*	Nil

Table-4: Phytochemical screening of *Anacardium occidentale* leaves

S.no	Tests	Aqueous extract	Ethanol extract
1.	Alkaloids	+	+
2.	Steroids	-	-
3.	Terpenoids	+	+
4.	Flavanoids	-	-
5.	Tannins	+	+
6.	Flavones	+	-
7.	Cardiac glycoside	+	+
8.	Proteins	+	+
9.	Carbohydrate	-	-
10.	Ninhydrin	-	-
11.	Lignin	+	+
12.	Inulin	+	+

(+) - Presence: (-) - Absence

Table 5: Minimal inhibitory concentration (MIC) of *Anacardium occidentale* extracts against diarrheal pathogens

Extracts	*E.coli sp.*	Salmonella sp	Klebsiella sp
Aqueous	500µg/ml	300µg/ml	450µg/ml
Ethanol	750µg/ml	600µg/ml	750µg/ml

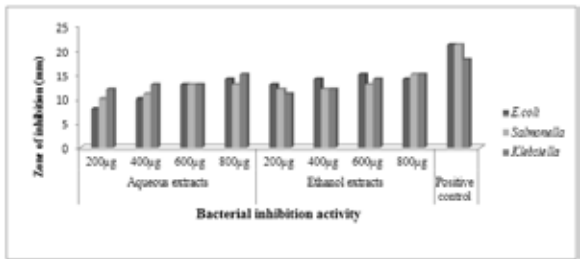

Figure.1: Bacterial inhibition potential of *Anacardium occidentale* extracts against diarrhea causing bacterial pathogens

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