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Study for antibacterial activity of cashew apple (*Anacardium occidentale*) extracts

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ABSTRACT

The present investigation was evaluated for potential antimicrobial activity of Anacardium occidentale, false fruit(cashew apple) extracts. Separately cashew apple were extracted with aqueous and ethanol solvents and their anti microbial activity was compared against few selected gram positive [Bacillus cereus (ATCC11778)]and gram negative bacteria [Klebsiella pneumoniae (ATCC11298)] by disc diffusion technique .The study revealed the potential antimicrobial activity of different false fruit extract of the Anacardium occidentaleThe Preliminary phytochemical analysis indicated the presence of various phytoconstituents in all the tested extracts.

Key words: *Anacardium occidentale*, Cashew apple extract, antimicrobial activity, Phytochemical screening, triterpenoids.

INTRODUCTION

Medicinal plants used in India for centuries as an important therapeutic source for treating a variety of ailments and has been found to be immense global importance.

There is a reluctance observed in accepting herbal remedies by modern system because of the lack of documentation regarding these called scientific validity and quality [1]**.** India is perhaps the largest producer of medicinal herbs and rightly called the 'Botanical garden of the world' .Medicinal herbs have been in use for thousands of years in one form or in the Indigenous system of medicines like Ayurveda, Siddha and Unanai.[2]

The present study aimed at evaluating the *in vitro* antimicrobial activity of various extracts of false fruit of *Anacardium occidentale*.

The microbial world comprises of micro-organisms which are microscopic in size. But these microorganisms have several features that are common to higher organisms. Bacteria, fungi (yeast and moulds) and microscopic algae are some of microorganisms. These organisms can be distinguished into two broad groups such as prokaryotes and eukaryotes. Eukaryotes contain nucleus and organelles (such as endoplasmic reticulum, golgi bodies, lysosome, mitochondrion and chloroplast) where as, prokaryotes lacks the above features.

The Anacardiaceae family consisting of several plants with immense pharmacological activity. Out of the plant *Anacardium occidentale* has been reported to have immense pharmacological and therapeutic activity. Various research work carried out has proved it to be used in various diseases like dermatitis , hyperglycemia ,antiviral anti inflammatory activity. It is traditionally accepted in Ayurveda to have antimicrobial activity.

Anacardium occidentale (Family:Anacardiaceae) is a native of tropical America , naturalized and cultivated throughout India especially near the coastal area, like Kerala from which the plant of interest is collected. *Anacardium occidentale* is used medicinally wherever it is found growing. The fruit and pericarp are officinal in Portugal. All parts of the plant like leaves, false fruit and bark have been traditionally used to relieve variety of ailments. *Anacardium occidentale* is a small tree with a short thick crocked trunk. Branches tereteglabrous .Leaves coriaceous ;10-15 by 3,8-7.5 cm, obviate or elliptic.

The fruit is acrid sweet, hot, digestible, aphrodisiac, anthelminthic; cures “vata” and“kapha”tumours, ascites, fever, ulcer, leucoderma and skin disease ,dysentery, piles, loss of apetite as mentioned in Ayurveda.

The bark is said to have alternative properties .The root is considered purgative and the fruit is mainly used as antidiarrhoel. The tar from the bark is used as a counter irritant .As an external application it has been recommended in leprosy, ring worm, and ostinate ulcers, it is powerfully rubifacient and vesicant and requires to be used with caution[3]. Keeping in mind about the adverse effect and toxicity of synthetic drugs. In the present study antimicrobial potential of alcoholic and aqueous extracts of false fruit of *Anacardium occidentale* have been evaluated along with preliminary phytochemical analysis.

MATERIALS AND METHODS

Plant material: The false fruit was collected from our campus in the month of March-April.The false fruits were identified and authenticated by Department of Botany, Payyannur college .Payyannur,

Preparation of extracts : The fruits were cleaned, reduced the size by cutting to small parts and then it is dried under shade. It is coarsely powdered with the help of a blender .The coarse powder of fruit was then exhaustively extracted in a Soxhlet apparatus.

In this extraction process 250 grams of dried powder was extracted by using soxhlet extraction process with 500ml of Ethyl alcohol and distilled water and chloroform(99:1) as solvent separately .The extractive values are given in the Table no: 1. The extracts were concentrated by

distilling the solvent and preserved under refrigeration for further studies . The dry extracts obtained were subjected to various chemical tests to detect the presence of different phytoconstituents [4,5]

Prelimianary phyto chemical analysis : Preliminary Phytochemical Analysis of the extracts was done to identify the chemical constituents present in the extracts prepared specifically as follows in Table No 2:-

- Test for carbohydrates was done using Molisch reagent and Fehlings reagent.
- Test for glycoside was done by following Tollens test, Legals test, Born Traggers test.
- Test for triterpenoids was prformed by Hirschorn test.Libermann storch mora sky test and test for Tannins and Phenolic compounds were also performed.

Microorganisms: The micro organisms were procured from CEEAL analytical lab, Chennai.
The anti-microbial activity of the synthesized compounds was screened against the following micro organisms.

The following gram negative and gram positive strains were used for the study.

- BACTERIA

Gram-positive organism: *Bacillus cereus* (ATCC11778)

Gram negative organism: *Klebsiella pneumoniae* (ATCC11298)

Medium: Nutrient agar medium (hi-media laboratories, India) is used as the media for the study of anti-bacterial activity.

The composition of the medium (gm/l):- Peptic digest of animal tissue 5.00g, Beef extract 3.00g, Sodium chloride 5.00g, Agar 15.00g.

In vitro Studies
Preparation of test extracts for antimicrobial activity study : The solutions of test compounds (ethanolic and aqueous extacts) were prepared at the concentration of 1 mg/ml by dissolving in Dimethyl formamide in a stoppered specific gravity bottle and stored in a refrigerator. The solution was removed from refrigerator one hour prior to their use and allowed to warm at room temperature. Similarly the standard drug solution of Ciprofloxacin in Dimethyl formamide was prepared at the concentration of 100µ/ml.

Determination of minimum inhibitory concentration
Tube dilution Technique
0.1gram (100mg) of dried evaporated aqueous and alcoholic extract were dissolved in 100ml of Dimethyl formamide giving final concentration of 1 mg/ml and both the extracts were tested for MIC by tube dilution method. [6,7]

Procedure: The procedure used for determining minimum inhibitory concentration is tube dilution method. Increasing order of extracts are added in to series of culture tubes as shown in Table no 3,containing nutrient agar medium inoculated with test micro organism, and the final volume is made up to 10ml using the nutrient agar medium . One tube is control contains no extract under examination. **.** The tubes were then incubated at 28+/-1^{o}C for 48 hours. The tubes were observed for growth of microorganism by observing the turbidity produced.

Antimicrobial Study

The cup-plate agar diffusion method was adopted to assess the antibacterial activity of the prepared extracts [8,9]

0.5 ml of standardized bacterial stock suspensions (10^{8}-10^{9}) colony – forming units per ml was thoroughly mixed with 50 ml of sterile nutrient agar. 20 ml of the inoculated nutrient agar were distributed into sterile Petri dishes. The agar was left to set and in each of these plates 4 cups, 10mm in diameter, were cut using a sterile cork borer No.4and the agar discs were removed.

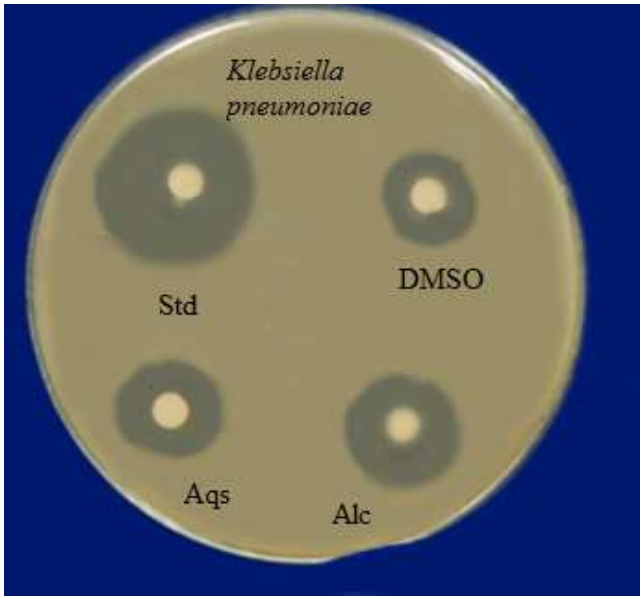
To each of the cups, test solutions of the respective extracts(1mg/ml)., ciprofloxacin(standard)and Dimethyl formamide(control) were added aseptically. The plates were then incubated at 28+/-1^{o}C for two days. After this the Petri plates were observed for the antibacterial activity and zone of inhibition was measured(figure no 1) . The experiment was repeated thrice and the average values are present in Table.No.4

RESULTS AND DISCUSSION

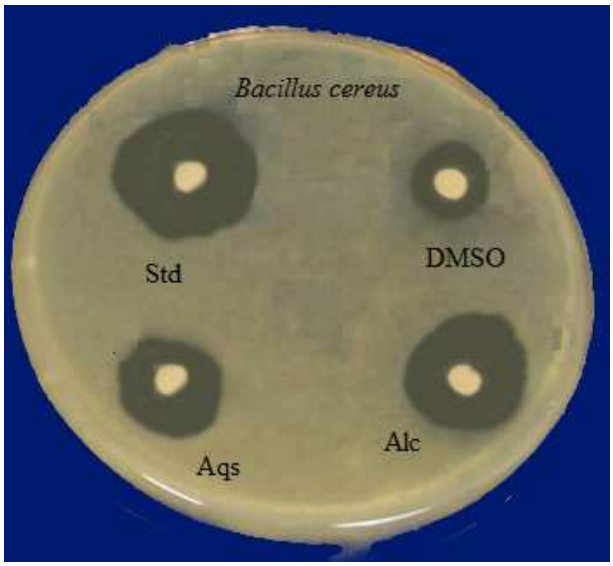
Preliminary phytochemical screening of alcoholic extract revealed the presence of steroids ,glycosides ,tannins ,phenolics and triterpenoids ,while aqueous extract showed the presence of steroids,glycosides ,carbohydrates,flavanoids and saponins The results are reported in Table no2.

As shown in Table no 3 and 4 the alcoholic and aqueous extract of false fruit of *Anacardium occidentale* displayed significant antimicrobial properties. Aqueous extract showed MIC as 0.08 mg/ml for *Bacillus cereus* and 0.09 mg/ml for *Klebsiella pneumoniae* and the Alcoholic extract showed MIC as 0.06 mg/ml for *Bacillus cereus* and 0.08 mg/ml for *Klebsiella pneumonia.*

By cup diffusion method both the extracts showed a significant zone of inhibition in comparison with standard drug ciprofloxacin (100µg/ml) .Alcoholic extract showed 26mm zone of inhibition against *Bacillus cereus and* 28mm zone of inhibition against *Klebsiella pneumoniae* and aqueous extract showed 24mm zone of inhibition against *Bacillus cereus* and 22mm zone of inhibition against *Klebsiella pneumoniae* .



a)showing zone of inhibition for *Klebsiella pneumonia*



b) showing zone of inhibition for *Bacillus cereus*

Figure 1: Showing results of Cup Plate Diffusion method

Table No 1 Extractive Values

Sl no	Extract	Percentage yield
1	Aqueous	1.08%
2	Alcoholic	1.28%

Table no 2: Qualitative phytochmical analysis of plant extract

Chemical Tests	Ethanol Extract	Aqueous Extract
Steroids	+	+
Glycosides	+	+
Carbohydrates	-	+
Flavanoids	-	+
Tannins and Phenolics	+	+
Triterpenoids	+	-
Saponins	-	+

(+): Shows the presence of the given chemical constituent.
(-): Shows the absence of the given chemical constituent

Table No 3 The protocol for evaluation of MIC-tube dilution technique

Sl no	Amount of extract per (ml)	Amount of medium(ml)	Total volume of solution (ml)	Concentration of extract in final solution (mg/ml)	Turbidity Produced			
					Aqueous extract		Alcoholic extract	
					Bacillus cereus	*Klebsiella pneumoniae*	*Bacillus cereus*	*Klebsiella pneumoniae*
1	0.1	9.9	10	0.01	+	+	+	+
2	0.2	9.8	10	0.01	+	+	+	+
3	0.3	9.7	10	0.01	+	+	+	+
4	0.4	9.6	10	0.01	+	+	+	+
5	0.5	9.5	10	0.01	+	+	+	+
6	0.6	9.4	10	0.01	+	+	-	+
7	0.7	9.3	10	0.01	+	+	-	+
8	0.8	9.2	10	0.01	-	+	-	-
9	0.9	9.1	10	0.01	-	-	-	-
10	1.0	9.0	10	0.01	-	-	-	-

+ Turbidity present, - Turbidity absent

Table no 4:- Antimicrobial activity of alcohol and aqueous extract of *Anacardium occidentale* false fruits.

Sl no	Test	Zone of Inhibition (mm)	
		Bacillus cereus	*Klebsiella pneumoniae*
1	Alc extract	26	28
2	Aqs extract	24	22
3	Dimethyl formamide	7	9
4	Standard	30	32

Invitro Antimicrobial studies:-
Alcoholic and aqueous extract of false fruit of *Anacardium occidentale* displayed significant antimicrobial properties. Both the extracts were tested for MIC by tube dilution method in which the microbial growth can be identified by turbidity produced.And the anti microbial activity is tested by cup diffusion method.

Aqueous extract showed MIC as 0.08 mg/ml for *Bacillus cereus* and 0.09 mg/ml for *Klebsiella pneumoniae* and the Alcoholic extract showed MIC as 0.06 mg/ml for *Bacillus cereus* and 0.08 mg/ml for *Klebsiella pneumoniae* .

By cup diffusion method both the extracts showed a significant zone of inhibition in comparison with standard drug ciprofloxacin 100µg/ml,

Phytochemical analysis of the crude extracts revealed presence of flavanoids,tannins ,triterpenoids and phenolic compounds ,and these components are responsible for the anti microbial activity of the extracts.These secondary metabolites serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores.Phenolic compounds shown to be toxic to microorganisms The site(s) and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity [10] In addition, some authors have found that more highly oxidized phenols are more inhibitory [11,12]The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins [13]

Flavanoids :Flavonoids are also hydroxylated phenolic substances but occur as a C_6-C_3 unit linked to an aromatic ring. Since they are known to be synthesized by plants in response to microbial infection [14], it should not be surprising that they have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls, as described above for quinones. More lipophilic flavonoids may also disrupt microbial membranes [15]

Tannins**:**"Tannin" is a general descriptive name for a group of polymeric phenolic substances capable of tanning leather or precipitating gelatin from solution, a property known as astringency,their relative antimicrobial action can be related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins, etc. They also complex with polysaccharide [16].

CONCLUSION

In conclusion the traditional claim of false fruit of Anacardium occidentale as an antimicrobial have been confirmed as the false fruit extract displayed activity against the micro organism used in the study .Further studies to isolate and reveal the active compound(s) contained in the crude extract of Anacardium occidentale and to establish the mechanism (s) of action are required to be done in future.

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