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ANTIOXIDANT AND ANTIMICROBIAL ACTIVITY USING DIFFERENT EXTRACTS OF *ANACARDIUM OCCIDENTALE* L.

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ABSTRACT : The present study highlights the investigation of antioxidant and antimicrobial property of different extracts of seed coat and leaf of cashew nut (*Anacardium occidentale* L.). The antioxidant activity was determined by the 2, 2- diphenyl -1 picryl hydrazyl (DPPH) method. Maximum activity was observed in acetonic extract of *Anacardium occidentale* leaf which was 52.50% (1000µg/ml). The antimicrobial activity had been tested for the plant parts using its aqueous, acetone and ethanol extracts against two Gram-positive human pathogenic bacteria like *Micrococcus luteus* (lab culture), *Staphylococcus aureus* (MTCC96), four Gram-negative human pathogenic bacteria *Salmonella typhi* (ATCC12600), *Klebsiella pneumoniae* (MTCC109), *Escherichia coli* (MTCC1687), *Pseudomonas aeruginosa* (MTCC733). The ethanol extract of the seed coat of *Anacardium occidentale* L. were most efficacious against all the test organisms with zone of inhibition ranging from 12.0-34.0 mm, and the acetonic extract of the leaf sample of *Anacardium occidentale* L. was also active against all the test organisms with zone of inhibition ranging from 12.0-28.0 mm.

Keywords: *Anacardium occidentale* L, *Anti-microbial & antioxidant activity*, Human pathogens, Agar-well diffusion.

INTRODUCTION

The intent of the present contemplation matches the recent concept for ethnopharmacology, which is a "Study involving number of academic disciplines of the consistent physiological action of plant, animal and other matter used in innate medicines of past and present cultures" (International Society of Ethnopharmacology, 2005). Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, oxygen-centred free radicals and other reactive oxygen species that are continuously produced *in-vivo*, result in cell death and tissue damage. Oxidative damage caused by free radicals may be related to aging and diseases, such as atherosclerosis, diabetes, cancer and cirrhosis (Halliwell and Gutteridge, 1984). The degenerative diseases associated with aging include cancer, cardiovascular disease, immune-system decline, brain dysfunction and cataracts (Ames et al., 1993). As carcinogenic properties have been reported for some synthetic antioxidants, recent research on the potential applications of natural antioxidants from spices and herbs, for stabilizing foods against oxidation, have received much attention (Aruoma et al., 1996).

The aggrandize in resistance of microorganisms due to multifarious use of commercial antimicrobial drugs encouraged scientists to scrutinize for new antimicrobial substances from various antecedents comprising medicinal plants (Karman *et al*., 2003). Various studies have looked at the antimicrobial activity of plant extracts. Considering the antimicrobial activity of *Anacardium occidentale* L. leaf and seed coat (skin of cashew nut). *Phyllanthus amarus* for its anti-hyperlipidemic activity (Van Holthoon, 2000) also indigenously for its hepatoprotective, antidiabetic, antihypertensive, analgesic, anti-inflammatory and antimicrobial properties (Adeneye *et al.,* 2006) analysis were determined on terpens, alkaloids, lignans, flavonoids and tannins in *Phyllathus* species (Vongvanich et al. 2000). *Ocimum sanctum* L. (Labiatae) is commonly known as "holy basil", 'Tulsi', Cock *et al* 2008 reported the antimicrobial activity of *O. sanctum* leaves against bacteria and yeast. *Coleus forskohlii* is especially connoted for cardiovascular diseases appending hypertension; congestive heart failure and angina. *Mentha longifolia*, wild mint is a notorious acknowledged medicine, chiefly used for respiratory ailments.

In the traditional civilization, one of the plants which have been used for ethno medical purposes is *Anacardium occidentale* L. (cashew nut), belonging to Anacardiaceae family, and a native of Brazil, having great economic and medicinal value and which is composed of some 60 to 74 genera and 400 to 600 species.

The leaves and seed coat of cashew tree has not been used as extensively as compared to many medicinal herbs or trees. It is not used as live stock feed for cattle also. Since the leaves and seed coat were not used effectively for any economical purposes, this study was undertaken to study its properties and ways to make it an economically important resource. *Anacardium occidentale* L. leaves, stem and bark extracts are utilized widely for the treatment of diarrhea, dysentery and colonic pain. (Bilcalho B, 2001). It has also been reported to possess anti-diabetic, anti-bacterial, anti-inflammatory and anti-ulcerogenic (Akinpelu DA, 2001). The leaves are also used in Brazil for eczema, psoriasis, scrofula, dyspepsia, genital problems, and venereal diseases, as well as for impotence, bronchitis, cough, intestinal colic, leishmaniasis, and syphilis-related skin disorders. The seed coat and the shell that remains after the extraction of the nut are used as fuel for burning purposes.

This project is an attempt to explore the hidden potential of the seed coat and leaves to inhibit plant and animal pathogenic micro organisms and methods to enhance its activity, if found, to make it an economically useful product. The study reported that the bark extract revealed *in-vitro* antimicrobial activity against 13 of 15 microorganisms tested (O. O. Igbinosa *et al.,* 2009). The plant produces many resources and products of importance.

Antibiotic and other drugs when used in excess cause damaging effect to the health. But plant sources are comparatively less harmful to the human health. Hence plant resources are widely used for medicinal purposes. Here in this report the antifungal and antibiotic properties of cashew nut is been studied by taking plant fungal pathogens and human bacterial samples and studying the antagonistic activity of cashew seed coat and leaf extracts by agar diffusion method given by Kirby-Bauer (1966).

MATERIALS & METHODS

Sample collection & Extraction

Anacardium occidentale L. leaf and cashew skin coat were collected from cashew plantation areas of Elaikadambur, Ariyalur district, Tamilnadu, India. The seed coat and the leaves were washed with running tap water and shade-dried for six days before they were powdered and kept in an airtight container prior to solvent extraction. 1 gram of the powdered seed coat sample and leaf samples were weighed and were soaked in 10 ml of water, ethanol (100%) and acetone and were kept over an orbital shaker at 150 rpm at room temperature for 48 hours. The acetone and ethanol extract were filtered with Whattman No. 1 filter paper, the residue discarded, and the filtrate was evaporated to semi solid state on a rotary evaporator at 40ºC. Since water is a non-volatile solvent, aqueous extract was lyophilized. The residue was suspended or dissolved in 1 ml of Phosphate Buffer Saline (pH 7.4). This was centrifuged at 5000 rpm for 5 minutes and the supernatant was collected and stored at 4°C in airtight bottle until further use.

Measurement of Antioxidant Activity

The antioxidant activity of the crude methanol extract of the plant *Anacardium occidentale* belongs to Anacadiaceae**,** family, the antioxidant activity were determined on the basis of their free radical scavenging activity was measured *in vitro* by using of the stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) . A solution of DPPH (0.1 mM) in acetone was prepared, and DPPH was added to tested solution with solvents at different concentrations (100-700 µg/mL). After 30 min; the absorbance was measured at 517 nm using Beckman spectrophotometer. The percentage of free radical scavenging abilities at different concentrations was determined. D-Ascorbic acid was used as a standard. The DPPH absorbs at 517 nm, and its concentration is reduced by the existence of an antioxidant. The method described by Hatano *et al.* (1988); Bhuiyan *et al.* (2009) was used as per reference, from the difference in absorbance on DPPH; the percentage of inhibition was calculated as a function of antioxidant activity.

% radical scavenging activity	=	**absorbance of blank - absorbance of scavenging activity sample**	**X 100**
		absorbance of blank	

Test organisms & cultures

Cultures were obtained from the culture collection centre. (Centre for Advanced Studies in Botany, Guindy, Chennai, Tamilnadu) of which two were Gram-positive human pathogenic bacteria *Micrococcus luteus, Staphylococcus aureus* (MTCC96) and other four Gram-negative human pathogenic bacteria *Salmonella typhi* (ATCC12600), *Klebsiella pneumoniae* (MTCC109), *Escherichia coli* (MTCC1687), *Pseudomonas aeruginosa* (MTCC733) the organisms were brought MTCC and ATCC.

Anti microbial sensitivity test

The agar well diffusion method was used to screen the antimicrobial activity. In vitro antimicrobial activity was screened by using Nutrient agar obtained from Himedia laboratories ltd (Mumbai). The Nutrient Agar plates were prepared by pouring 15-20 ml of molten media into sterile Petri plates; they were allowed to solidify for 5 minutes. Then 100 µl of the inoculum suspension was swabbed uniformly and it was allowed to dry for 5 minutes. Wells, with diameter of 7 mm, were cut on the surface of the plates (NA); different concentrations of extracts (50, 75 and 100 µl) were loaded into the wells. In NA the compound was allowed to diffuse for 60 minutes and the plates was kept for incubation at 37°C for 24 hrs.

Then the % inhibition was calculated by the following equation:

RESULTS & DISCUSSION

The results confer the utility of *Anacardium occidentale* L. plant leaf extract in developing a novel broad spectrum antimicrobial agent.

Antioxidant activity

Anacardium occidentale leaf crude extract was assessed for their capacity to prevent the formation of 1, 1-diphenyl-2-picrylhydrazide (DPPH) peroxide radicals in a peroxy-generating system as described. The antioxidant activity of the plant leaf extract shown in Table. 1. The standard D-Ascorbic acid which shows the activity of 61.71% at a minimum concentration of 6 µg/mL. When compare to standard *Anacardium occidentale* 52.50% (1000 µg/mL).

Antimicrobial activity

The 3 different concentrations (50, 75 and 100 µl) of the seed coat and leaf extracts showed varying degree of antimicrobial activities.

M.luteus and S.aureus (MTCC96) showed 11 mm and *E.coli* (MTCC1687) showed 10 mm zone of inhibition with 100 µl of the *Phyllanthus amarus* aqueous leaf extract whereas the *Anacardium occidentale* L. aqueous leaf extract of 50 µl inhibited *M.luteuse* with 13 mm, *S.aureuse* (MTCC96) with 10.5 mm, *E.coli* (MTCC1687) with 12.9 mm and *P.aeuroginosae* (MTCC733) with 11 mm zone of inhibition.

Whereas the ethanol extract of P.amarus of 100 µl inhibited *S.typhi* (MTCC12600) with 15 mm and others with 10 -14 mm zone of inhibition comparatively 75 µl of the ethanol extract of *A. occidentale* seed coat and leaf inhibited *E.coli* (MTCC1687) with 13 mm and 33 mm respectively.

100 µl acetone extract of *P.amarus* inhibited *M.luteus* with 19 mm and the rest with 11-16 mm zone of inhibition. Whereas 100 µl of acetone extract of *A.occidentale* L. seed coat inhibited *M.luteus* and *K.pneumonia* (MTCC109) with 24 mm and the others with 19-21 mm zone of inhibition; and the leaf extract inhibited *P.aureginosa* (MTCC733) with 28 mm and the others with 19- 26 mm zone of inhibition.

Phytochemical analysis of *A. occidentale* L. Nuts: A variety of rich secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides and volatile oils are present in plants in general. The ethanolic extracts of *A. occidentale* L. shows the presence of various phytochemical compounds such as triterpenoids, phenolics and volatile oils. Ethyl acetate extracts exhibited a different combination of phytochemicals, phenolics, volatile oils, xanthoprotein and carbohydrates. Acetone found to be effective in dissolving the phytochemicals since many different compounds like triterpenoids, phenolics, volatile oils, flavonoids, xanthoprotein and carbohydrates were observed. However, Acetone, acted as good solvent for flavonoid extraction. The obtained results are in accordance with the reports of Tedong *et al.,* that phytochemical analysis of *A. occidentale* L. revealed the presence of alkaloids, polyphenols and saponins.

Table 1: Antioxidant activity of *Anacardium occidentale*

S.No.	Concentration (µg/mL)	% Inhibition
1.	200	15.55
2.	400	22.22
3.	600	34.72
4.	800	41.66
5.	1000	52.50

The ethanolic extract contains high amount of terpenoids, phenols and volatile oils (Tedong *et al.,* 2006), and any of these compounds could be responsible for the inhibition of micro organisms.

The results from the present study showed that the two extract (acetone and ethanol) of *Anacardium occidentale* displayed efficacious antimicrobial activity against the considered 6 human pathogenic bacterial strains. As seen from the results (Table no. 2 & 3), both the extracts have showed a broad spectrum of activity. When the two crude extracts were compared with each other and with that of a standard antibiotic, Amikacin (30 mcg/ml), ethanolic extract of seed coat and acetonic extract of leaf samples was seen to possess a greater potential, compared to the other extracts.

Table 2: List of pathogenic bacteria and diseases

Name of the pathogenic Organisms	Disease
Micrococcus luteus (Laboratory culture)	Septic shock, septic arthritis, endocarditis, meningitis, and cavitating pneumonia
Staphylococcus aureus (MTCC96)	Food poisoning and toxic shock syndrome and can cause bumble foot
Salmonella typhi (ATCC12600)	Typhoid and paratyphoid fever
Klebsiella pneumoniae (MTCC109)	Notably pneumonia, septicemia ankylosing spondylitis.
Escherichia coli (MTCC1687)	Cholecystitis, Bactremia, Cholangitis, Diarrhea
Pseudomonas aeruginosa (MTCC733)	Urinary tract infection, ventilator associated pneumonia

Table3: Vulnerability pattern of human pathogens to *Anacardium occidentale* seed coat and leaf extract (50 µl)

	Diameter of zone of inhibition (mm)					
	Concentration of Seed coat extract (3 mg / 50 µl)			**Concentration of leaf extract** (3 mg / 50 µl)		
Organism	Aqueous extract	Acetone	**Ethanol**	Aqueous extract	**Acetone**	Ethanol
M. luteus (gram+)	13	20	**24**	13	**21**	18.5
S. aureus (gram +)	10	16	**21**	10.5	13	14
E. coli (gram -)	12	17	**26**	10	13	12
K.pneumoniae (g-)	-	18	12	-	12	12
S. typhi (gram -)	-	14	**23**	-	12	16
P.aeruginosa (gram)	-	**24**	15	10	23	13

Acetone extract of the *Anacardium occidentale* L. leaf proved effective against all the test organisms with a maximum zone of inhibition of about 28 mm/100 µl against *Pseudomonas aeruginosa* (MTCC733). Zone of inhibition for *E.coli was* 17 mm/100 µl obtained for acetonic extract of anacardium leaf, was found to be greater compared to the chloroform + hydrochloric acid (13 mm/100 µl) extract of *Andrographis paniculata* (Soma roy *et al.,* 2009) and higher than that given by methanolic extract of leaves of *Acacia nilotica* (15 mm), *Tinospora cordifolia* (14 mm) and *Sida cordifolia* (15 mm) (S.Satish *et al.,* 2008). Whereas, ethanol extract of the seed coat proved effective against all the test organisms with a maximum zone of inhibition for *E.coli* (MTCC 1687) 34 mm/100 µl and for *Pseudomonas aeruginosa* (MTCC733) 31 mm/100 µl, which is even greater than the zone obtained from the leaf sample of *Anacardium occidentale* L.

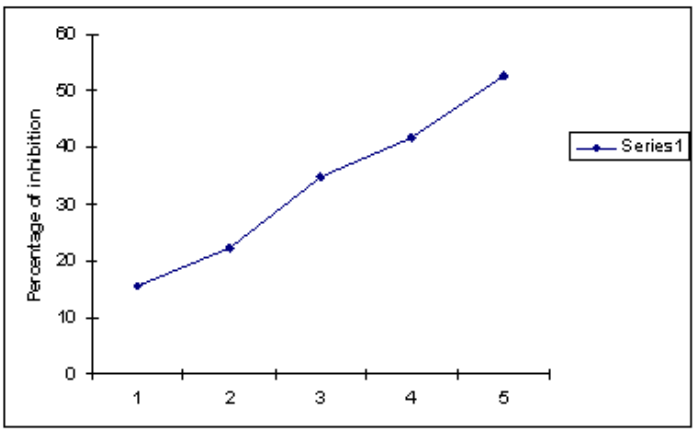
This is one of the reports on the analysis of antioxidant and antimicrobial activity of crude compounds from different parts of *Anacardium occidentale* L. The antioxidant property was only checked with leaf extract whereas for the antimicrobial activities were tested against human pathogenic bacteria with both leaf and seed coat extracts. The results confer the utility of this plant extract in developing a novel d broad spectrum antimicrobial agent.

Table 4: Vulnerability pattern of human pathogens to *Anacardium occidentale* seed coat and leaf extract (75 µl)

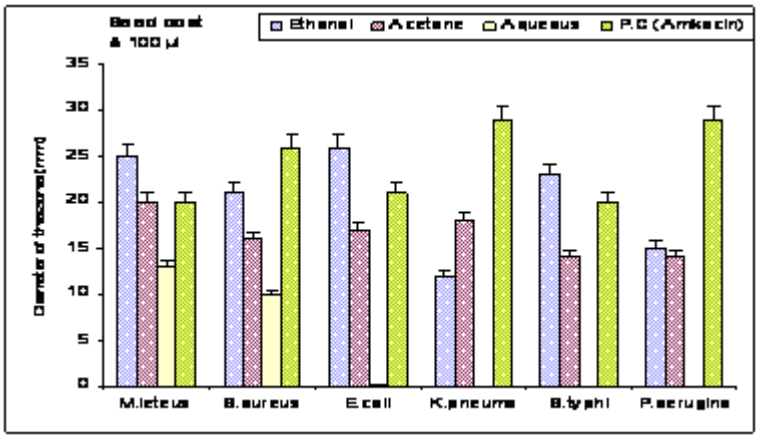
Organism	**Diameter of zone of inhibition (mm)**					
	Concentration of Seed coat extract (4.5 mg / 75 µl)			**Concentration of leaf extract (4.5 mg /75 µl)**		
	Aqueous extract	Acetone	Ethanol	Aqueous extract	Acetone	Ethanol
M. luteus (gram+)	14	22	**26**	13	**23**	19
S. aureus (gram +)	10	18	21	11	14	16
E. coli (gram -)	13	18	**33**	11	14.5	13
K.pneumoniae (g-)	-	21	14	-	13	14
S. typhi (gram -)	-	16	25	-	13	18
P.aeruginosa (g-)	-	16	21	13	**24**	17

Table 5: Vulnerability pattern of human pathogens to *Anacardium occidentale* seed coat and leaf extract (100 µl)

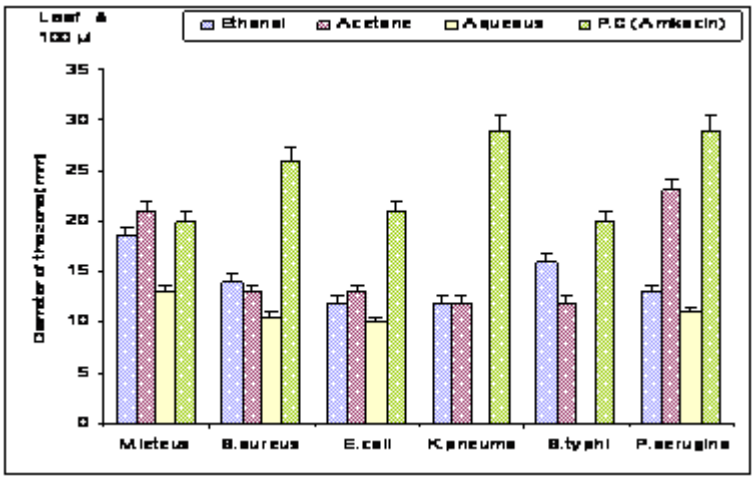
	Diameter of zone of inhibition (mm)					
	Concentration of Seed coat extract (6 mg / 100 µl)			**Concentration of leaf extract(6 mg / 100 µl)**		
Organisms	Aqueous extract	Acetone	Ethanol	Aqueous extract	Acetone	Ethanol
M. luteus (gram+)	14	24	**28**	13	24	21
S. aureus (gram +)	11	21	27	-	16	19
E. coli (gram -)	13	21	**34**	11	17	14
K.pneumoniae (g-)	-	24	18	-	14.5	18
S. typhi (gram -)	-	22	27	-	14	20
P.aeruginosa (g -)	-	19	**31**	14	**28**	19



Graph1. Antioxidant activity of *Anacardium occidental* leaf



Graph 2.Vulnerability pattern of human pathogens to *Anacardium occidentale* seed coat (100 µl)



Graph 3. Vulnerability pattern of human pathogens to *Anacardium occidentale* leaf (100 µl)

Conclusion

In this study supports the use of these plants in traditional medicine to treat various ailments like stomach complaints, wound infections and intestinal disorders etc. *A.occidentale* L. exhibited apical antimicrobial activity in seed coat and leaf sample which is expected to be a renowned source of antimicrobial agents for the future endeavours.

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