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Research Article

Phytochemical and biological activirty of *Anacardium occidentale L.* from Visakhapatnam region, Andhra Pradesh, India.

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Abstract: The present study describes the preliminary phytochemical screening, quantitative analysis of phytochemicals and evaluation antimicrobial properties of *Anacardium occidentale* L. The results showed that the phytochemical contents varied significantly between the sample extracts. Total phenolic content of the *Anacardium occidentale* plant significantly from 10 – 60 mg/g sample. Extracts of Anacardium exhibited appreciable inhibition on all human pathogens screened. Results indicated that the green synthesized AgNPs showed good antibacterial effect against studied microflora. *Anacardium occidentale* AgNPs presented antibacterial activity, especially against Gram-negative bacteria, in concentrations where no significant cytotoxicity was observed. The antimicrobial properties of silver nanoparticles of *A. occidentale* derived from the presence of a polyphenol, anacardic acid and other compounds, tatrols and tanins. Therefore, it is suggestive of the fact that extracts could be used as an development of nutraceuticals and functional foods for the control, management and treatment of health disorders, antibiotic formulation especially in poor communities.

Keywords: Green Synthesis, Silver Nanoparticles, Antibacterial Activity.

Introduction

Infectious diseases are responsible for 45% of deaths in low income countries and 50% of premature death worldwide. Among the deaths caused by microorganisms, bacterial infections account for 70% of cases (Walsh, 2003). Unfortunately, the emergence of antibiotic-resistant bacteria has put an end to this wave of optimism (Adejuwon *et al.,* 2011). In this context, medicinal plants have been considered excellent candidates for research because of their high antibacterial potential [Hildebrand, 2006; Allaker, 2010].

Throughout the ages, nature has provided humans with source of the essential of life, including food, medicines and raw materials for the manufacture of clothing and shelters. Man began to explore the resources of every type on earth for their potentialities to cure ailments and interpreted their effects wisely in terms of knowledge in different branches of science prevailing at that time. Medicinal plants are of great importance to the health of individuals and communities. Medicinal plants contain physiologically active components which over the years have been exploited in the traditional medical practices for the treatment of various ailments

Anacardium occidentale L. (Family Anacardiaceae), is a multipurpose tree of the tropics which attains a height of about 15m. They grow on relatively dry soil in nature but in cultivation grow well in the tropical rain forest. The cashew tree produces many products and resources. According to Duke (2001), the bark and leaves of *A. occidentale* are used medicinally; the shell oil around the nut is used medicinally and for industrial applications in the plastic and resin industries for its phenol content; the nut has international appeal and market value as a food source, especially in the beverage industry.

The main aim of this work was to

I. To evaluate *A. occidentale* as the new sources of antimicrobial agents, that can be used study against drug resistant bacteria.
II. To determine the major classes of phytochemicals and bioactivities of the crude extracts of targeted medicinal plants.
III. Preliminary phytochemical and physicochemical screening of the plant to assure the quality, safety, and efficacy of this formulation.
III. Determine the antibacterial activity of plant extracts against a diverse range of pathogens by using agar well diffusion method, minimum inhibitory concentration (MIC).

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Materials and Methods

Solvents and chemicals:

All chemicals were purchased from Qualigens fine Chemicals, Mumbai and S.D. fine chemicals, Mumbai. All culture media components and antibiotics used in this study were procured from Hi Media, Mumbai, India.

Medicinal plants

Anacardium occidentale L. selected by using previous work done, folklore reports, based on cost effectiveness, ease of availability and was collected during the flowering periods from G. Madugula, Visakhapatnam District, Andhra Pradesh and identified with the help of Gamble, "Flora of the Presidency of Madras" and later verified by comparison with the authenticated specimens available in the herbariums of NBRI, Lucknow and the Department of Botany, Andhra University, Visakhapatnam. The plant was then identified and confirmed by a taxonomist working at Andhra University.

All the fresh and healthy plant parts of leaves were collected locally, washed with running tap water and double distilled water respectively for several times to remove all the dust and unwanted visible particles, cut into small pieces and dried at room temperature and then shade dried for 3 to 5 days, and was powdered by using mechanical grinder and then stored for further investigations.

Preparation of plant extract

Soxhlet apparatus is used for extraction. 100grams of ground weighed material of fine coarse powder was successively extracted by different solvents of hexane, chloroform and methanol, in a specific sequence based

on increasing polarity. The solvents were removed under reduced pressure and controlled temperature by rotary evaporator. The extracts were dried and stored in a clean glass bottle and kept at 2-8^{0}C for antimicrobial screenings and further investigation.

Physicochemical parameters

The physicochemical parameters were used to analyse the purity and quality of the extract. The extracts were evaluated for parameters like loss on drying, total ash and extractive values according to the methods recommended by the World Health Organization (WHO, 1998). Ash value is useful in determining authenticity and purity of drugs and these values are important for quantitative analysis. Studied plant materials were evaluated by following physicochemical parameters.

Loss on Drying (LOD):

5 grams of air-dried plant material (extract) was placed in a crucible of silica. The extract was spread in a thin uniform layer. The crucible was then placed in the oven at 105^{0}Celsius for 4 hours and cooled in a desiccator to room temperature and weight of the cooled crucible plus powder was noted.

$$\% \text{ Loss on Drying (LOD)} = \left(\frac{\text{Weight}_{(\text{crucible + dry})} - \text{Weight}_{(\text{dry})}}{\text{Weight}_{(\text{crucible + drug})} - \text{Weight}_{(\text{crucible})}} \right) \times 100$$

Extractive values:

Accurately weighed 5 grams of extract placed in the glass stoppered conical flask and macerated with 25 mL of methanol (95%) for 6 hours with frequent shaking, mixture allowed to stand for 18 hours. After completion of 18 hours, filtered rapidly taking care not to lose any solvent. Transferred the filtrate in tared flat bottom porcelain dish. Filtrate was evaporated to dryness on water bath, dried at 105 degree Celsius for 6 hours cooled in desiccator for 30 minutes and weighed. Calculated the content of extractable matter in milligrams per gram of air-dried material.

$$\% \text{ of Extractive Value} = \left(\frac{\text{Extract obtained}}{\text{Weight of powdered sample}} \right) \times 100$$

Total Ash value:

5 gm of extract was taken in tarred platinum crucible. Extract was spread in fine even layer at bottom of the platinum crucible. This platinum crucible with material was kept in muffle furnace for ignition at high temperature. Temperature of furnace increased gradually up to 450 degree Celsius. The material was kept at this temperature for 6 hours till complete ignition of extract occurred, that is till complete white colored ash was obtained, intermittent weighing was also done. Crucible was then taken out from furnace, cooled and weighed. The total ash was calculated by subtracting the weight of crucible with ash of extract after ignition from weight of crucible with extract powder before ignition. Percentage of total ash was calculated with reference to air dried extract.

$$\% \text{ of Total Ash Value} = \left(\frac{\text{Weight of total ash}}{\text{Weight of crude extract taken}} \right) \times 100$$

Preliminary phytochemical investigation

The extracts of phytochemical analysis for identification of bioactive chemical constituents were carried out by using standard methods of Harbone, 1984.

Quantitative determination of phytochemicals in studied Plants

The quantity of various phytochemicals presented in studied medicinal plants were determined as follows

Alkaloids

Alkaloid were determined according to the method by Edeoga *et al.,* with slight modifications as follows: 2.5 g of the plant material powder was extracted using 100 mL of 20% acetic acid in ethanol. The solution was covered for almost 4 hours. Filtrate was concentrated to 25 mL. Concentrated ammonium hydroxide was added stepwise to attain precipitation. The whole solution was kept as such so that precipitate will settle. Collected precipitate was washed with dilute ammonium hydroxide and finally filtered. Filtrate was discarded and pellet obtained was dried and weighed [Edeoga *et al.,* 2005].

Flavonoids

The flavonoids were determined by the method according to Kumaran [Kumaran. and Karunakaran, 2007], with slight modifications. 5 mL of 2 % aluminium trichloride in methanol with the same volume of aqueous plant extract. The absorbance was then read at 420 nm after one hour of incubation against a blank sample of 5 mL extract solution with 5mL methanol without aluminium trichloride. The total flavonoid was determined using a standard curve of pyrocatechol ranging from 0.5 to 16 mg/100 mL, and the results expressed as mg/g pyrocatechol equivalent.

Total Phenolic content:

Total phenolic content was determined using Folin-Ciocalteau reagent and gallic acid as the standard according to the method by Rasineni *et al.,* (2008). About 500 mg of milled plant material powder was weighed and homogenized in 10 mL of n-hexane. The homogenate was centrifuged at 10,000 × g for 20 minutes and the supernatant was used in the determination of total phenols as follows. About 0.5 mL of Folin-Ciocalteau 2 N reagent was added to 2.5 mL of the supernatant and then 2 mL of 10% sodium carbonate in ethanol. The mixture was incubated for 5 minutes at 20 degree Celsius and then the absorbance read in triplicates at wavelength of 650 nm.

Saponins

Saponin was determined as per the method by Edeoga *et al.,* (2005) with slight modifications: 10 gram of sample was mixed with 100 mL of 20% aqueous methanol. The mixture was kept for 4 hours on water bath shaker at 55 degree Celsius. Filtrate was again extracted in same manner. The combined extract was concentrated to 40 mL over water bath at 90^0 Celsius. Concentrate obtained was transferred into a separating funnel and 10 mL of diethyl ether was added to it. After shaking vigorously aqueous layer was recovered and ether layer was discarded. The process was repeated and to the aqueous layer n-butanol was added. The whole mixture was washed in separating funnel twice with 10 mL 5% of aqueous NaCl. Upper part was retained and heated in water bath until evaporation. Later it was dried in oven to a constant weight.

Tannins

The tannins were determined as follows: 2 gram of plant powder was extracted thrice in 70% acetone. After centrifuging the sample supernatant was removed.

Different aliquots were taken and final volume to 3 mL was adjusted by distilled water. The solution after vortexing were mixed with 1 mL of 0.016M $K_3Fe(CN)_6$, followed by 1 mL of 0.02 M $FeCl_3$ in 0.10 M HCl. Vortexing was repeated and the tubes were kept as such for 15 min. The stabilizer was prepared in the ration of 3:1:1 of water, H_3PO_4 and 1% gum arabic, and then 5 mL was added to the mixture followed by re-vortexing. Absorbance was measured at 700 nm against blank. Standard curve was plotted using various concentrations of 0.001 M gallic acid [Gurib-Fakim, 2006].

Antimicrobial Studies

Test organisms:

The antibacterial activity of the hexane, chloroform and methanol crude extracts was tested against both Gram positive, Gram negative bacteria and fungal organisms. Three gram positive bacteria including *Bacillus subtilis* MTCC441, *Enterococcus faecalis* MTCC439 *Staphylococcus aureus* MTCC737, three gram negative bacteria including *Escherichia coli* MTCC443, *Proteus vulgaris* MTCC426 and *Pseudomonas aeruginosa* MTCC1688 and three fungal strains of *Candida albicans* MTCC227, *Trichophyton mentagrophytes* MTCC7687 and *Epidermophyton floccosum* MTCC613 were used in the study.

***In vitro* antimicrobial assays:**

The antibacterial activity of the methanol extracts was determined using the agar well diffusion method of Adeniyi, *et al.,* [1996] with modification. Nutrient agar plates were seeded with 0.2mL of an overnight culture of each bacterial isolate. The seeded plates were allowed to set and a standard cork borer of 6 mm diameter was used to cut uniform wells on the surface of the agar. The wells were then filled with 100 µL of each extract at a concentration of 100 mg/ml. Ciprofloxacin at 1 mg/ml was used as positive control. The plates were incubated at 37^0 C for 24h after which the diameter of zones of inhibition were measured.

Result and Discussion

Anacardium oxidantalis yielded the crude extracts of hexane (3.09), chloroform (2.15) and methanol (10.9). Methanol was an extremely efficient extractant with an average of 16% extracted.

Physicochemical parameters

Physicochemical parameters were following those mentioned in Ayurvedic Pharmacopoeia of India. The percentage of loss of weight on drying, total ash were obtained by employing standard methods of analysis.

Loss on Drying

The test for loss on drying determines both water and volatile matter. *Anacardium oxidantalis,* Loss on drying value at 105^0C was fond to be 7.59 %

Extractive Values

Plant extractives were analysed to estimate the percentage yield of individual extracts and found that, the yield was abundant in methanol rather than chloroform and hexane. Due to the high polarity of methanol most of the chemical constituents of extracts would be dissolved in it and thus percentage yield was increased tremendously than other solvents. *Anacardium oxidantalis* found 21.04% extractive in total. Methanol extracts were found high yielding.

Total Ash values

In the evaluation of crude extracts, total ash value is particularly important in the evaluation of purity of drugs i.e., the presence or absence of foreign inorganic matter such as metallic salts and silica [Musa, *et al.,* 2006]. *A. occidentale*s yield ash comtent of 7.56%. The amount and composition of ash remaining after combustion of plant material varies considerably according to the part of the plant, age, treatment etc. The constituents of ash also vary with time and from organ to organ. Ash usually represents the inorganic part of the plant. The total ash method is designed to measure the total amount of material remaining after ignition.

Preliminary phytochemical investigation

After performing the analysis *Anacardium oxidantalis*, following phytochemical groups of alkaloids, coumarins, flavonoids, phenols, saponins, tannins and terpenoids were detected as present in the studied medicinal plants in different proportions and cardiac glycosides, glycosides, quinones were absent.

Alkaloids

The alkaloid contents were examined in plant extracts and expressed in terms of atropine equivalent as mg of AE/gram of extract (the standard curve equation: y = 0.006x – 0.003, R2 = 0.997). Alkaloid contents found to be 37.85 ± 0.11 in hexane, 27.89 ± 0.52 chloroform where as 44.25 ± 0.07 AE/gram of extract in methanol.

Flavonoids

Flavonoids are class of secondary plant metabolites with significant antioxidant and chelating properties. Antioxidant activity of flavonoids depends on the structure and substitution pattern of hydroxyl groups [Sharififar *et al.,* 2008]. All the studied plant extracts have high concentration of flavonoids contents found to be 43.85 ± 2.11 in hexane, 16.89 ± 0.53 chloroform where as 16.78 ± 0.15 mg of RU/g of extract in methanol.

Total Phenolic Contents

The total phenolic contents in the examined plant extracts using the Folin-Ciocalteu's reagent is expressed in terms of gallic acid equivalent (the standard curve equation: y = 7.026x – 0.0191, r2 = 0.999). The values obtained 09.19 ± 0.02 in hexane, 18.39 ± 0.06 in chloroform where as 57.47 ± 0.75 mg of GAE/g of extract in methanol.

Saponins

Saponins were present in studied plants showed characteristic foaming properties in aqueous solution. The values obtained 30.85 ± 0.22 in hexane, 27.89 ± 0.52 in chloroform where as 28.25 ± 0.07 percent of extract in methanol.

Tannins

The tannins contents were examined in plant extracts using the Folin-Ciocalteu's reagent is expressed in terms of gallic acid equivalent. The values obtained 13.85 ± 0.11 in hexane, 17.89 ± 0.53 in chloroform where as 77.35 ± 0.07 mg of GAE/g of extract in methanol.

Antimicrobial activity

The invention and development of antibiotics are most powerful and successful achievements of modern science and technology for the management of infectious diseases. However, the rate of resistance of pathogenic

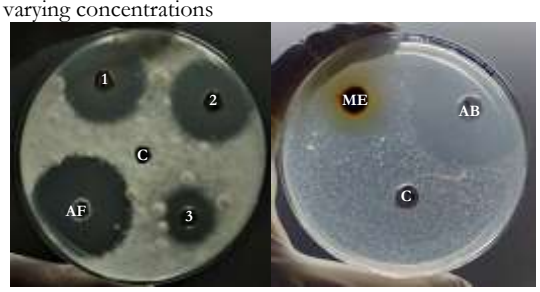
microorganisms to conventionally used antimicrobial agents is increasing with an alarming frequency [Nair, R. and Chanda, S., 2005; Neogi, U. *et al.,* 2008].

Table 1: Antimicrobial activity of *Anacardium oxidantalis* methanol extracts

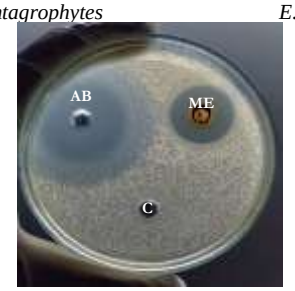
Plant Name	100 mg/ml	MIC mg/ml
B. subtilis	10 ± 0.15	50 ± 1.01
E. faecalis	08 ± 0.06	100 ± 1.14
S. aureus	11 ± 0.09	50 ± 1.25
P. vulgaris	10 ± 0.15	75 ± 1.00
E. coli	16 ± 0.06	50 ± 0.25
P. aeruginosa	15 ± 0.20	50 ± 0.25
T. mentagrophytes	16 ± 0.17	25 ± 0.11
E. floccosum	09 ± 0.23	100 ± 0.10
C. albicans	17 ± 0.12	25 ± 0.10

*zone of inhibition in mm; 6mm borer size

Figure: Antimicrobial activity of methanol extracts at varying concentrations



T. mentagrophytes *E. coli*



P. aeruginosa

1, 2, 3-100,250,500mg/ml;
AB/AF- Ciprofloxacin/Fluconazole;
C -DMSO; 50µl per well; 6mm cup borer size.

Table 1 depict the antibacterial activity of the extract of *Anacardium occidentale* leaves respectively. Cashew methanol extracts show significant antibacterial activity but to varied degrees. The relative measurement of the antibacterial activity of the methanolic extract of *A. occidentale* reveal significant statistical disparity between the leaves and the bark extracts (t value= 4.24, p0.05). Table 1 shows the effect of different concentrations of methanolic extract on the antiinflammatory activity of *A. occidentale*. No apparent variation was found between the different concentrations examined (F value = 4.40, P>0.05).

The present study showed that *A. occidentale* extracts are effective inhibitors of microbial growth. All extracts showed varying levels of activity against both Gram positive and Gram-negative bacteria as well as the fungal yeast. The methanol extract of the leaves of *A. occidentale* was highly active against *E. coli* and showed a zone of inhibition nearly with positive control antibiotic (Ciprofloxacin). The extracts also showed considerable activity against *P. aeruginosa* and *T. mentagrophytes*. The activity against *E. coli* therefore justifies the traditional use of *A. occidentale* in the treatment of diarrhea and is similar to the work of Kudi *et al.,* [1999]. The methanol extract of the leaves of *A. occidentale* was more active against all test bacteria than the hexane and chloroform extracts. This may be due to the ability of methanol to extract a wider range of antibacterial principles than the aqueous solvent [John Brittos, 2001]. The phytochemical analysis of *A. occidentale* revealed the presence of alkaloids and tannins. Goncalves *et al.,* [Goncalves, 2005] had earlier reported the presence of tannins, alkaloids, saponins, terpenes and flavonoids in *A. occidentale* leaves. Thus the antimicrobial activity of the extracts on the test organisms may be due to the presence of the above phytochemical components.

Conclusions

More research is required to fully establish the usefulness of this plant, *A. occidentale* to humanity as a source of drugs for the alleviation of illnesses caused by micro and macro-organisms. There is need for the development of new antimicrobials to combat the problem of the spread of resistance more importantly from natural sources as this delays the emergence of resistance. It can be concluded that the present results provides a basis for the development of new pharmaceuticals from *A. occidentale*.

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