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IN-VITRO ANTI-BACTERIAL POTENCY AND PHYTOCHEMICAL ANALYSIS OF *ANACARDIUM OCCIDENTALE* L. LEAF AGAINST SOME GASTROINTESTINAL TRACT INFECTION-CAUSING BACTERIA

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Keywords:

Anacardium occidentale, Anti-bacterial activity, Gastrointestinal tract infection

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ABSTRACT: Bacterial infections are one of the prominent causes of health problems and mortalities around the world. Medicinal plants are a rich source of antimicrobial agents and provide a safer and cost-effective way of treating bacterial infections. The objective of the present investigation was to evaluate the anti-bacterial potential of petroleum ether and methanol extracts of *Anacardium occidentale* L. leaf. The anti-bacterial potential of *A. occidentale* L. leaf was tested against gastrointestinal tract infection-causing bacteria such as *Escherichia coli, Shigella flexneri, Enterococcus faecalis, Vibrio cholariae, Staphylococcus aureus, Klebsiella pneumoniae,* and *Salmonella typhi* by using the agar well diffusion method. Streptomycin was taken as the reference antibiotic. The results indicated that petroleum ether extract of *A. occidentale* L. exhibited the highest zone of inhibition against *Shigella flexneri* (26±0.75) and least against *Staphylococcus aureus* (11±1.32). In contrast, methanolic extract exhibited the highest zone of inhibition on *Vibrio cholariae* (20±1.41) and least against *Escherichia coli* (11±0.21). The preliminary phytochemical analysis revealed the presence of alkaloids, glycosides, tannins and polyphenolic compounds, flavonoids, saponins, steroids, triterpenoids, proteins and amino acids, carbohydrates, etc. The petroleum ether and methanolic extracts were subjected to a TLC study, and the R_f value of different spots was observed in various solvent systems. The activity of the plant extracts is due to the presence of different phytoconstituents, which may be utilized for the development of newer therapeutic agents against gastrointestinal tract infections.

INTRODUCTION: Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value.

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Recently, the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led to an investigation of the antimicrobial activity of medicinal plants [1]. Medicinal plants are natural sources of compounds that can be used against several diseases today [2]. The discovery of many natural and synthetic drugs is remarkable progress in the field of medicine, which has been made by the advancement in Science and Technology [3].

One of the most important therapeutic discoveries of the 20th century is antibiotics that had high effectiveness against serious bacterial infections. However, only one-third of the infectious diseases known have been treated from these synthetic products [4]. The extracts of several plants have been used as therapeutic agents. Many drugs presently prescribed by physicians are either directly isolated from plants or are artificially modified versions of natural products [5]. These medicines are safe and environmentally friendly. According to the WHO, about 80% of the world's population relies on traditional medicine for their primary health care [6]. Moreover, the increasing use of plant extracts in the food, cosmetic and pharmaceutical industries suggests that, in order to find active compounds, a systematic study of medicinal plants is very important.

Since ancient times, humanity used various natural materials as a source of medicines, and plants have probably always had the most important role to play in medicine and public health [7]. As a result of centuries of accumulated knowledge and experience, people often used plants for treatment purposes until the development of modern medicines. Unfortunately, the preservation of this valuable knowledge is in danger because its transmission between older and younger generations is not always assured [8]. Therefore, documentation of the local knowledge through ethnobotanical studies is important for the conservation and utilization of biological resources. For these reasons, ethnobotanical studies have become increasingly valuable for the development of healthcare in today's era.

With the increasing commercialization of herbal products, there was a need for the scientific community to introduce a quality control system for plant-based medicines. The Government of India introduced an amendment in 1964 to the Drug and Cosmetics Act 1940 to have stringent quality control on Ayurvedic, Siddha, and Unani drugs. Progressively, the development of standards for the identity, purity, and strength of single drugs and formulations assumed importance for the effective enforcement of the provisions of the Act. The raw materials in herbal medicines are expected to conform to uniform standards. It is necessary to identify the drugs, determine their quality, and detect adulterations. Standards for quality control of herbal formulations or preparations are based on pharmacognostical, physicochemical, phytochemical, and biological parameters.

The test plant material - *Anacardium occidentale* L. (cashew) is a small tree, often branched from near the base, belonging to the family Anacardiaceae. Flowers yellow with pink stripes, in terminal panicles. Fruit a nut, reniform, grayish brown, seated on fleshy yellowish or reddish hypocarp. Cultivated and run wild, especially along the sea coast. *A. occidentale* L. (cashew) has been scientifically explored for varied pharmacological and phytochemical investigations. This plant has hidden potentiality for its medicinal and economic importance [9-11].

The roots are considered purgative. The bark has alternative properties and is used along with inflorescence for the treatment of snakebite. The bark and leaves are useful in odontalgia and ulitis. The gum from the bark is recommended in leprosy, ringworm, corns, and obstinate ulcers. Fruits are acrid, sweet, thermogenic, aphrodisiac, trichogenous and anthelmintic, and are useful in skin diseases, dysentery, haemorrhoids, and anorexia.

They are used for preventing hair loss and to increase the growth of hair [12]. The leaves of this plant contain tannins, β-sitosterol, ethylgallate, hyperoside, methylgallate, leucocyanidin, leucodel-phinidins, myricetin, agathisflavone, robustaflavone, quercetin, kaempeferol, apigenin, 3-O-rhamnoside and quercetin-3-O-glucoside [9].

In the present study, petroleum ether and methanolic extracts of *A. occidentale* leaf have been evaluated for their anti-bacterial activities as well as the presence of different phytochemicals.

MATERIALS AND METHODS:

Collection and Identification of Plant Material: The plant *Anacardium occidentale* L. was collected from Utkal University campus, Bhubaneswar, Odisha, in the month of April 2015. Identification of the voucher specimen (BOTUU-1156) was done by following 'The Flora of Orissa' [12]. The voucher specimens were deposited in the herbarium of the Post Graduate Department of Botany, Utkal University, Vani Vihar, Bhubaneswar, Odisha.

Chemicals and Reagents: All the chemicals and reagents used for the study were of analytical grade obtained from S.D Fine Chem. Ltd., Mumbai.

Method of Preparation of Plant Extract: The leaves were collected in bulk amount, washed in running tap water, dried under shade, and made to coarse powder form. The leaf powder was successively extracted with petroleum ether and methanol for 48 h using Soxhlet extractor [13]. The dilute extracts were concentrated by distilling off the solvent under reduced pressure. The extracts were kept in desiccators for further use. The dried extracts were weighed, and their percentage in terms of the dry weight of the plant material was estimated by the following formula:

Percentage of extract yield = (weight of dried extract/weight of dried plant material) × 100

Microbial Strain: Seven gastrointestinal disease-causing bacteria, including strains of both gram-positive and gram-negative (*Escherichia coli, Shigella flexneri*, *Enterococcus faecalis, Salmonella typhi*, *Vibrio cholariae, Klebsiella pneumoniae, Staphylococcus aureus*) were selected for bioassay study. The bacterial pathogens such as *Shigella flexneri* (MTCC-9543), *Klebsiella pneumoniae* (MTCC-109), and *Staphylococcus aureus* (MTCC-1430) were procured from Microbial Type Culture Collection Centre (MTCC) and Gene Bank, Chandigarh, India. The remaining bacterial species were provided by the Post Graduate Department of Microbiology, OUAT, Bhubaneswar, Odisha.

Microbial Culture Media: For anti-bacterial susceptibility test, the extracts were tested on solid (Agar-Agar) media on petri plates by applying the agar well diffusion method. For bacterial assay nutrients agar (NA) (28 gm/L) [(HIMEDIA), REF-M001-500G, LOT-0000145979] was used for developing surface colony growth. The suspension culture for bacterial cell growth was done by preparing nutrient broth [(HIMEDIA), (REF-M088-500G, LOT- 0000154058)]. All the prepared media were sterilized by autoclaving the media at 121°C for 20 minutes.

Preparation of Fresh Culture: The nutrient agar medium was prepared and dispersed in clean test tubes to prepare slants (5 ml in each test tube). The test tubes were plugged with cotton and sterilized for 30 min. After sterilization, the test tubes were kept in an inclined position (45º) for solidification. The test organisms were transferred to the agar slants from the procured pure cultures with the help of an inoculating loop in an aseptic condition. The inoculated slants were then incubated at 37 ºC for 24 h to assure the growth of test organisms. These fresh cultures were used for the sensitivity test.

Preparation of Test Plates: Nutrient agar media were transferred to the sterile petridishes in sterile area. About 30 ml of the media was poured into each petridishes in such a way as to keep a uniform depth of approximately 4 mm. The petridishes were rotated several times, initially clockwise then anticlockwise. Then the plates were swabbed (sterile cotton swabs) with 8 h old broth culture and kept preserved for applying samples.

Preparations of Test Samples: The stock solution of the plant extract was prepared at a concentration of 20 mg/ml in DMSO and different concentrations were made by following serial dilution method.

Agar Well Diffusion Method: Agar well diffusion method was followed to determine the zone of inhibition of microbes in nutrient agar. Wells (10 mm diameter and about 2 cm apart) were made in each of these swabbed plates using sterile cork borer. About 50 µl of this plant extracts were added with micropipette into the wells and allowed to diffuse at room temperature for 2 h. Streptomycin (0.5 mg/ml) was taken as the standard drug. The plates were incubated at 37 °C for 18-24 h for the growth of bacterial pathogens. Triplicates of each treatment were maintained, and the experiment was repeated thrice; for each replicates the readings were taken in three different fixed directions, and the average values were recorded.

Measurement of the Zone of Inhibition: The diameter of the inhibition zone (mm) was measured with the help of a transparent scale. The anti-bacterial activity of petroleum ether and methanol extracts having concentration 20 mg/ml followed by concentrations of 10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml were tested against the bacterial strains.

Preliminary Phytochemical Analysis: The extracts were subjected to preliminary phytochemical investigation in order to detect the presence of different metabolites in the plant. A very minute

amount of plant extracts were taken and treated with various chemical reagents as per the test procedure [14, 15].

TLC Profiling: The extracts were subjected to Thin Layer Chromatography (TLC) analysis by using different solvent systems. After development, TLC plates were sprayed with reagent (sulfuric acid: methanol-5:95), dried, and spots were observed, and R_f value was calculated [16].

RESULTS AND DISCUSSION:

Anti-bacterial Activity: The leaf extract of the test plant subjected to anti-bacterial screening against seven bacteria, namely *Escherichia coli, Shigella flexneri, Enterococcus faecalis, Vibrio cholariae, Staphylococcus aureus, Klebsiella pneumoniae,* and *Salmonella typhi,* causing gastrointestinal disorders in human being. The antibactericidal effect of the plant extracts were investigated in the form of an inhibition zone against different pathogens. The results of anti-bacterial activity of the plant extracts revealed that most of the extracts have the potential to inhibit bacterial growth **Table 1**. The results indicated that petroleum ether extract of *A. occidentale* L. exhibited the highest zone of inhibition against *Shigella flexneri* (26±0.75) and least against *Staphylococcus aureus* (11±1.32). In contrast, the methanolic extract exhibited the highest zone of inhibition on *Vibrio cholariae* (20±1.41) and least against *Escherichia coli* (11±0.21). Surprisingly the methanol extract at the concentration of 1.25 mg/ml did not inhibit the growth of *E. coli*. The pet ether extract of the test plant showed broad-spectrum anti-bacterial activity in comparison to methanolic extract. It was interesting to note that the anti-bacterial activity of the extract was more potent at the highest concentration against all the tested strains.

Preliminary Phytochemical Analysis: The preliminary phytochemical investigation of the plant extracts revealed the presence of alkaloids, glycosides, and polyphenolic compounds, tannins, saponins, flavonoids, amino acids, steroids, triterpenoids, proteins, and carbohydrates **Table 2**.

TLC Profiling: The TLC study was carried out by using different solvent systems. The colour and R_f values of different spots are recorded (Table 3). Maximum 5 spots were observed by the TLC study of pet ether extract with the solvent system, n-hexane: acetone (75:25).

TABLE 1: ANTI-BACTERIAL ACTIVITY OF *ANACARDIUM OCCIDENTALE* LEAF EXTRACT

Test substance	Conc.	Diameter of the inhibition zone (mm)						
		Vibrio cholariae	*Shigella flexneri*	*Salmonella typhi*	*Escherichia coli*	*Enterococcus faecalis*	*Klebsiella pneumoniae*	*Staphylococcus aureus*
Streptomycin (RA)	0.5 mg/ml	36±0.81	32±1.03	33±0.63	24±1.29	31±0.36	30±1.43	35±0.46
Petroleum ether extract	20 mg/ml	24±0.46	26±0.75	21±1.21	22±0.53	21±0.68	20±1.24	21±0.81
	10 mg/ml	22±0.81	24±0.61	20±0.47	19±0.47	19±0.46	19±0.21	18±1.63
	5 mg/ml	21±0.94	23±0.81	18±1.41	18±0.4	17±0.47	16±0.48	15±0.43
	2.5 mg/ml	20±0.84	18±0.82	15±1.24	17±1.69	15±0.34	14±1.31	13±1.41
	1.25 mg/ml	18±1.24	16±0.24	14±1.62	13±0.23	13±1.34	13±0.38	11±1.32
Methanol extract	20 mg/ml	20±1.41	14±0.81	18±0.47	12±1.63	15±1.21	16±1.23	18±1.82
	10 mg/ml	18±o.81	13±0.471	16±0.46	11.66±0.94	14±0.461	15±0.48	16±0.47
	5 mg/ml	14±o.61	12±0.81	15±0.21	11.33±1.24	13±0.27	14±0.61	14±0.51
	2.5 mg/ml	13±o.21	11±1.24	14±1.21	11±0.21	11±0.52	12±0.21	13±1.35
	1.25 mg/ml	12±0.31	11±1.21	13±0.62	---	11±0.53	11±0.68	12±1.24

Result expressed as mean ±S.D (n=3), (---) denotes no zone of inhibition

TABLE 2: PRELIMINARY PHYTOCHEMICAL ANALYSIS OF *ANACARDIUM OCCIDENTALE* LEAF EXTRACT

S. no.	Chemical test	Petroleum ether extract	Methanol extract
1	Alkaloids	+	+
2	Glycosides	+	+
3	Flavonoids	+	-
4	Saponins	-	+
5	Steroids	+	-
6	Triterpenoids	+	+
7	Tannins and polyphenolic compounds	+	+
8	Proteins and amino acids	+	+
9	Carbohydrates	+	+

(+) indicating presence of chemical compounds; (-) indicating absence of chemical compounds

TABLE 3: TLC PROFILING OF *ANACARDIUM OCCIDENTALE* LEAF EXTRACT

Plant extract	Solvent system	Spot	Colour	R_f
Petroleum ether extract	n-hexane : acetone (75:25)	Spot 1	Pink	0.53
		Spot 2	Light green	0.28
		Spot 3	Dark green	0.25
		Spot 4	Light green	0.21
		Spot 5	Light green	0.19
	n-hexane : ethyl acetate (85:15)	Spot 1	Yellow	0.89
		Spot 2	Green	0.54
		Spot 3	Pink	0.27
		Spot 4	Light green	0.16
	Chloroform : methanol (74:26)	Spot 1	Pink	0.52
		Spot 2	Purple	0.28
		Spot 3	Light green	0.23
Methanol extract	n-hexane : acetone (74:26)	Spot 1	Light green	0.87
		Spot 2	Yellowish	0.63
		Spot 3	Light pink	0.59
	n-hexane : ethyl acetate (73:27)	Spot 1	Yellow	0.67

CONCLUSION: From the present study, it can be concluded that *Anacardium occidentale* is endowed with promising antimicrobial activity against bacterial strains causing gastrointestinal tract infection. The petroleum ether extracts of this plant showed more antimicrobial activity than methanol extract. The inhibitory effect of the leaf extracts of *Anacardium occidentale* against pathogenic bacteria used in the present study indicates that this plant can be a potential candidate for various drug developments for the treatment of ailments caused by most of the pathogens. It is hoped that further studies on chemical analysis of the plant may lead to the isolation of important bioactive molecules, which may be specific for the antimicrobial activity. The isolation of bioactive constituents and their structural elucidation can be done by using various modern chromatographic techniques.

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CONFLICTS OF INTEREST: All the authors have none to declare.

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