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Antibacterial Activity of Fruit Extract of *Anacardium occidentale* and Seed Extract of *Mangifera indica L.*

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Abstract: *Mangifera indica L. and Anacardium occidentale members of the Anacardiaceae family have gained the attension due to their therapeutic property. The present study evaluated the presence of phytochemicals and antibacterial activity of ethanolic and methanolic extraction of both plants. Phytochemical screening showed the presence of glycosides, saponin, flavonoid, tannin, alkaloid, steroid, phenolic compound, and triterpenoid in both solvent extracts. Gram-positive and Gram-negative organisms were found to be sensitive to the ethanolic and methanolic extraction of both plants. The MIC of mango seed extract was found to be 50 µg/ml and cashew fruit extract was 8 µg/ml. The study indicates the potential future application of mango seed & Cashew fruit extract as an antibacterial agent which would be an alternative to the current chemical antibacterial agent.*

Keywords: Mango Seed Extract, Cashew Fruit Extract, Antibacterial Activity, MIC

I. INTRODUCTION

The growing multi-drug resistance amongst microorganisms is a global concern nowadays. The majority of pathogenic organisms become resistant due to prolonged use of the same antibiotic compounds such as methicillin, vancomycin, isoniazid and rifampin, penicillin. (Russell et al.,2002) reported several antibiotic-resistant bacteria, including methicillin-resistant staphylococcus aureus (MRSA), vancomycin-resistant enterococci (VRE), multidrug-resistance in Mycobacterium tuberculosis (MDRTB) strains, and multi-drug resistant (MDR) Gram-negative bacteria. Multidrug-resistant bacteria are creating an unsafe environment for human health and also becoming a challenge to the medical field.

Thus extraction of phytochemicals from medicinal plants for the discovery of an effective and harmless alternative to chemically synthesized antibiotics can be used. Medicinal plants have been used in India for centuries to treat various pathogenic infections. Medicinal plants include Aloe, Tulsi, Neem, Turmeric, Ginger, Mango, Cashew, etc. The medicinal plant is any plant that contains a substance that can use for therapeutic purposes or the synthesis of drugs. Medicinal plants play a significant role in disease prevention (Sofowera et al.,2013). (Schiber et al., 2003) stated that phytochemicals prevent oxidative phosphorylation in bacteria thus preventing the growth of microorganisms and act as antimicrobial agent.

Mangifera indica Linnaeus and A*nacardium occidentale* are two important medicinal plants. These plants are members of *Anacardiaceae* family includes various flowering and fruit-bearing plants that possess different biochemical activities. The *Anacardiaceae* plants are distributed from tropical, subtropical to temperate regions. The *Mangifera indica L.* and *Anacardium occidentale* were reported to bear chief therapeutic properties. (Kittiphoom S. et al.,2012) reported that Mango seed contains a high level of proteins, fat, and antioxidants thus can be used as antimicrobial compounds or in cosmetics. (Bhagirathi et al.2018) reported that the Cashew plant is a potential source of bioactive compounds that improve disease conditions.

Medicinal plants are known to secret some types of chemicals called as secondary metabolites or phytochemicals (Ogunmefun et.al,2018). Phytochemicals are responsible for the medicinal properties of the plant. There are different types of phytochemicals present in the plant such as saponin, tannins (Makkar et.al 1992), alkaloids, glycosides, phenol

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(Slinkard K et.al 1977), and flavonoids (Anandan et.al 2017). (Bhagirathi et.al,2018) carried out antibacterial activity of Cashew fruit extract by using disc diffusion test and reported plant phytochemicals protect human health and metabolic conditions from numerous pathogens,

Phytochemicals inhibit peptidoglycan synthesis in cell wall, it damages bacterial membrane structure, affects the bacterial adhesion to the surface and also affects quorum sensing (Monte et.al.,2014).(Aiswarya. G etal.,2011) used Cashew apple extract to check antibacterial activity by using disc diffusion method and also shown that these plants can be used to treat various diseases. (El-Desoukeyet.al.,2020) used Mango peel extract to check its antibacterial and antifungal activity by using agar well diffusion method.

Many industries using Mango for the production of juices , jams , concentrates , dried fruits , fruits bars etc. The Mango seed are treated as waste after the use of Mango pulp in such industries. The Mango seeds comprise 17-22 % of total Mango fruits (Kittiphoom S. et al.,2012). Cashew nut industries are also using only nuts part of Cashew apple for the nut production. But most of the Cashew apple is left in the farm as agricultural waste which is creating lots of waste. The weight of the leftover Cashew apple is about 10 times of the harvested nuts (Attri, 2009).

If the study shows good results against bacteria, Mango seeds and Cashew apple can be utilized at the large scale production of antibacterial agent which is a natural source. Also there are so many other applications of Mango seeds and Cashew apple which industries can use to reduce solid waste.

II. Materials and Methods

2.1 Collection of Sample

Fresh fruits of *Mangifera indica L.* and A*nacardium occidentale* Were collected from nearby APMC marketplace, Panvel , India

2.2 Preparation of Mango seed Crude Extract

Mango seeds were collected, dried in oven at 40^0c for 24 hrs. and were grounded to fine powder. Methanol and ethanol were used as an extractant in the assays with ratio of 5:10 of plant material to the extractant. To prepare methanolic extraction 50 gm of Mango seed powder was soaked in 100 ml of methanol in round bottom flask. For ethanolic extraction 50 gm of Mango seed powder was soaked in 100 ml of ethanol in round bottom flask. The flasks were transferred to incubator shaker for 24 hrs. at 37 ° c. The extracts were filtered using Whatman no.1 filter paper and concentrated by drying in oven and the dry extract obtained was then used for further antibacterial study.

2.3 Preparation of Cashew Fruit Crude Extract

Cashew fruits were collected, washed properly, cut into small pieces and dried in oven at 40^0c for 24 hrs. and was grounded to fine powder. Methanol and ethanol were used as an extractant in the assays with ratio of 5:10 of plant material to the extractant. To prepare methanolic extraction 50 gm of Cashew fruit powder was soaked in 100 ml of methanol in round bottom flask. For ethanolic extraction 50 gm of Cashew fruit powder was soaked in 100 ml of ethanol in round bottom flask. The flasks were transferred to incubator shaker for 24 hrs. at 37 ° c. The extracts were filtered using Whatman no. 1 filter paper and concentrated by drying in oven and the dry extract obtained was then used for further antibacterial study.

2.4 Preliminary Phytochemical Screening

In the plant extracts , various phytochemical components were estimated by using standard methods. The presence of glycosides, saponin, flavonoid, tannin, alkaloid, steroid, phenolic compound, and triterpenoid were detected using standard tests.

2.5 Microorganisms

The antibacterial activity of plant extracts were evaluated against the 4 bacterial strains causing bacterial gastroenteritis. The three gram-negative bacteria i.e. *Escherichia coli, pseudomonas aeruginosa, salmonella typhi* and one gram positive bacteria i.e. *Staphylococcus aureus*. Bacterial species were collected from Hi-Media Laboratory,

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Thane, India. Each bacterial strain was subcultured , maintained on Muller Hinton agar plates at 4^0c and grown at 37^0c for use whenever required.

2.6 Antibacterial Assay of Plant Extracts

In-vitro antibacterial activity was evaluated for plants phytochemical extract by using agar well diffusion method.

2.7 Agar Well Diffusion Method

The sterile Muller Hinton agar was seeded with 0.1 ml of 10^8 CFU/ml bacterial cultures was then poured into sterile petri plates. Wells were made with sterile 6 mm Cork borer. Mango and Cashew solvent stock solution was then diluted to concentration 5mg/ml, 10mg/ml , 25mg/ml , 50mg/ml. The 0.1ml of each prepared concentration was poured into each well and plates were incubated at 37^0c for 24 hrs. Zone of inhibition was measured for each well.

2.8 Determination of MIC

MIC of the plant extract was measured using serial dilution method. The sterile Muller Hinton broth was mixed with mango seed extract and cashew fruit extract separately. Broth dilution was carried to prepare concentrations from 500 µg/ml to 0.1 µg/ml. The 0.1 ml of bacterial culture of 10^8 CFU/ml was added into the sterile broth test tube containing solvent extract. The tubes were incubated at 37^0c for 24 hrs. and lowest concentration that inhibit bacterial growth was observed.

III. Result and Discussion

3.1 Preliminary Phytochemical Screening

Qualitative phytochemical screening of *Mangifera indica L* revealed the presence of important phytochemical compounds such as glycoside , saponin , flavonoid , tannin and alkaloids. Qualitative phytochemical screening of A*nacardium occidentale* indicated the presence of glycoside , saponin , steroids ,phenolic compounds and triterpenoids.

Table 1: Preliminary Phytochemical Screening

Phytochemical compound	Test	Cashew fruit	Mango seed
Glycosides	Brontagers test	+	+
Saponin	Foam test	+	+
Flavonoid	Ammonia test	-	+
Tannin	Lead acetate test	-	+
Alkaloid	Mayer's test	-	+
Steroid	Liebermann-Burchard's test	+	-
Phenolic compound	Ferric chloride test	+	-
Triterpenoid	Test for triterpenoids	+	-

(+) : Shows presence of phytochemical compound
(-) : Shows absence of phytochemical compound

3.2 Antibacterial Assay of Plant Extracts :

The antibacterial activity of *Mangifera indica L.* and A*nacardium occidentale* plant extracts was evaluated by using Agar well diffusion method against standard drugs vancomycin and kanamycin. The antibacterial activity of both plant extracts was expressed in the term of the presence of zone of inhibition in diameter (in mm). Table 2 clearly shows that both plant extracts exhibit antibacterial activity against all tested organisms. Ethanolic plant extracts of *Mangifera indica L.* and A*nacardium occidentale* showed better results than methanolic extracts. Ethanolic mango seed extract showed the highest zone of inhibition against *E.coli (*24 mm) and the lowest zone of inhibition against *P.aeruginosa (*7 mm). The antibacterial activity of tested plant extract is attributed to the presence of phytochemicals in the extracts.

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Table 2: Zone of Inhibition of Bacteria

Fruit	Solvent Extract	Zone of Inhibition (mm)			
		E.coli	*S. aureus*	*P. aeruginosa*	*S. typhi*
Mango	Ethanol	24mm	23mm	10 mm	15 mm
	Methanol	22mm	19mm	8 mm	10 mm
Cashew	Ethanol	26mm	26mm	12 mm	13 mm
	Methanol	22mm	21mm	7 mm	8 mm
Control	Vancomycin(30µg/ml)	-	28	14	19
	Kanamycin (30µg/ml)	25	-	-	-

3.3 Minimum Inhibitory Concentration

The minimum inhibitory concentration of methanolic and ethanolic extract of *Mangifera indica L.* and A*nacardium occidentale* was determined against pathogenic bacteria. As shown in table no. 3 & 4. Results clearly shows that MIC of methanolic and ethanolic extract of cashew fruit and mango seed is 50 µg/ml & 200 µg/ml respectively.

Table 3: Minimum inhibitory concentration of cashew fruit extract.

	Methanolic Extract								Ethanolic Extract							
	Concentration of extract (µg/ml)								Concentration of extract (µg/ml)							
Isolates	**3**	**6.5**	**12.5**	**25**	**50**	**100**	**200**	**300**	**3**	**6.5**	**12.5**	**25**	**50**	**100**	**200**	**300**
E.coli	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
P. aeruginosa	+	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-
S. salmonella	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-
S. aureus	+	+	+	-	-	-	-	-	+	+	+	-	-	-	-	-

(+):*Turbidity: present* , (-):*Turbidity: Absent*

Table 4: Minimum inhibitory concentration of mango seed extract.

	Methanolic Extract								Ethanolic Extract							
	Concentration of extract (µg/ml)								Concentration of extract (µg/ml)							
Isolates	**3**	**7**	**12.5**	**25**	**50**	**100**	**200**	**300**	**3**	**7**	**12.5**	**25**	**50**	**100**	**200**	**300**
E.coli	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-
P. aeruginosa	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	-
S. salmonella	+	+	+	+	+	-	-	-	+	+	+	+	-	-	-	-
S. aureus	+	+	+	+	+	-	-	-	+	+	+	+	-	-	-	-

IV. Conclusion

The present study conclusively indicated the presence of antibacterial activity of ethanolic and methanolic extract of cashew fruit and mango seeds. The antibacterial activity of both plants is attributed to the presence of various phytochemical compounds in the plant. In addition , study also indicated that there is no significant difference in the activity of ethanolic and methanolic extract. .*Mangifera indica L.* and A*nacardium occidentale* should be further studied to elucidate their therapeutic potential in clinical application.

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