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IN-VITRO SCREENING OF ANTIBACTERIAL EFFICACY OF *CADABA FRUTICOSA* (L.) AGAINST COMMON HUMAN PATHOGENS

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ABSTRACT

Infectious diseases caused by Bacteria and Fungi are a growing global threat today, accounting for 50% of the death in tropical countries. Though, conventional drugs provide effective therapy against these infections, development of Multi Drug Resistance, high cost and adverse side effects of synthetic chemotherapeutics urged the researchers to search for an alternate, safe and natural remedy from enormous range of medicinal plants which is the treasure of India. In the current investigation, *Cadaba fruticosa* (L.) which is called as *Vizhuthi*, *Adamorinika*, *Chikondi* and *Piluka* in Tamil and *Indian Cadaba* in general is extracted with alcohol, chloroform and water and screened for its antibacterial activity against the common pathogens *viz.*, *S.aureus*, *Pseudomonas*, *Bacillus* sp., *E.coli*. All the three extracts showed promising results in the control of pathogens. Alcohol fraction showed 17.4 mm zone of inhibition (ZOI), aqueous extract showed 16.8 mm zone of inhibition (ZOI) and chloroform fraction showed 11.9 mm zone of inhibition at 1600 µg concentration / well. *S.aureus* (17.4 mm) and *E.coli* (14.5mm) were highly sensitive to alcohol fraction. *Pseudomonas* was highly susceptible to chloroform fraction (14.7mm). In general, *Bacillus* was least sensitive to all the fractions.

KEYWORDS: Infectious diseases, antibacterial activity, Alcohol fraction, chloroform fraction, zone of inhibition (ZOI).

INTRODUCTION

All over the World, microorganisms like bacteria and fungi are predominantly responsible for the increased infections in human beings and animals. According to the World Health Organization (WHO), infectious diseases are the number one cause of death worldwide and account for 50% of the deaths in tropical countries. Though, conventional drugs provide effective therapy against these infections, microbes possess the genetic ability to develop resistance against several synthetic chemotherapeutics and create immense problems in treatment which is the global threat today. In addition, the host experiences many adverse effects by consuming these drugs [Adwan *et al.,* 2008). Finding out an alternate, safe and natural remedy is the need of the hour to overcome such abnormal and grave situation. Nature is an abundant first rate store-house consisting of an enormous range of medicinal plants. According to WHO 80% of the world's population relies on plant derived medicines which serves as, first line of defense in maintaining health and combating many diseases (Bolu *et al.,* 2004). Major parts of the traditional therapies involve the use of plant extracts or their bioactive components. Plant based natural constituents can be derived from any part of the plant like bark, leaves, roots, fruits, seed, fruit rind, etc (Anjana Sharma *et al.*,2009). Medicinal plants have been used as an exemplary source for centuries as an alternative remedy for treating human diseases because they contain numerous active constituents of therapeutic value. However, most species of higher plants have less surveyed for their chemical or biologically active constituents. Hence, in the present investigation, stem of *Cadaba fruticosa* (L.) which is called as *Vizhuthi, Adamorinika, Chikondi* and *Piluka* in Tamil and *Indian Cadaba* in general is extracted with alcohol, chloroform and water, having the objectives of screening its antibacterial activity against the common pathogens *viz., S.aureus, Pseudomonas Bacillus* sp., *E.coli* and analyzing the Phytochemical status of the *Cadaba fruticosa*(L.) extracts.

MATERIALS AND METHODS

Collection of Plant Materials

The healthy plants of *Cadaba fruticosa(L.)* was collected from in and around areas of Tiruchirappalli District, Tamilnadu, South India. The collected plants were authenticated at Rapinat Herbarium, St. Joseph College, Tiruchirappalli – 2 and deposited in the same place for future references.

Preparation of Leaf Powder

The collected *Cadaba fruticosa(L.)* plant material was surface sterilized with sterile distilled water to remove the dirt and soil particles adhered on them. Stem pieces were sorted out cut into small pieces and shade dried at room temperature (32°C) for two weeks. The shade dried stems were pulverized into a coarse powder and stored at room temperature for further use.

Extraction of Plant Material

Organic fractions *viz.,* Chloroform, Alcohol and aqueous extracts of *Cadaba fruticosa(L.)* Plant stems were obtained by standard hot and cold extraction methods. (Harborne *et al.*, 1999).

Phytochemical Analysis

Preliminary phytochemical analysis for biologically active phytoconstituents like quinones, flavonoids, tannin, coumarins, sugars, steroids, phenols, terpenoids, anthroquinones etc., of the plant extracts were carried out using standard methods described by Harborne *et al.*,(1999).

Antibacterial Assay

Characterized and identified bacterial cultures such as, *pseudomonas* sp *S.aureus, Bacillus* sp. and *E.coli* were obtained from Doctor's Diagnostic Centre, Tiruchirappalli, Tamilnadu. The bacterial strains were maintained in Nutrient agar medium and utilized for the current study.

Based on well diffusion method (Perez, 1990) the antibacterial assay was performed. Stock solutions of aqueous extract and organic fractions were made by dissolving 40 mg of extracts in 1 ml of Dimethyl sulfoxide (DMSO) and sterilized by using sortorious syringe filter. Sterile Petri plates containing 20 ml of Mueller Hinton agar medium were seeded with 0.01ml of 12-18 hours old test bacterial culture with calibrated loop (Himedia) and lawned evenly using sterile cotton swabs. Wells were made using well cutter and added with one drop of sterile agar at the bottom of the well to seal it. All the extracts and fractions were added at different concentrations *viz*., 400µg, 800µg, 1200µg, and 1600µg. Incubation was made at 37^{o}C for 24 hours. The assessment of antibacterial activity was based on the measurement of diameter of the inhibition zone formed around the well, using Hi-media scale. Streptomycin sulphate 100µg and DMSO 30 µl were used as positive and negative controls respectively. Triplicates were made for all the experiments.

RESULTS AND DISCUSSIONS

Table 1: Phytochemical analysis of *Cadaba fruticosa(L.)*

S. No.	Phytocompounds	Aqueous	Alcohol	Chloroform
1.	Steroids	-	-	-
2.	Triterpenoids	-	-	-
3.	Sugars	+	-	-
4.	Alkaloids	+	+	+
5.	Phenolic compounds	+	+	+
6	Flavonoids	+	+	+
7	Saponins	_	-	-
8.	Tannins	-	-	-
9	Anthroquinones	–	-	-
10	Coumarin	+	+	+

+ Present; – Absent

Table. 2: Antibacterial activity of aqueous extracts of *Cadaba fruticosa(L.)* against pathogens by Agar well diffusion method.

S.no	Conc of plant extracts in µg/ well	Zone of Inhibition in mm/ Test organisms			
		S.aureus	***Bacillus* sp.**	***E.coli***	***Pseudomonas* sp**
1	400µg	9.6	6.0	7.9	8.2
2	800µg	10.6	6.9	9.8	9.4
3	1200µg	13.6	7.4	11.0	10.4
4	1600µg	16.8	8.3	13.5	14.7
5	Positive control	20.1	20.1	20.1	20.1
6	Negative control	-	-	-	-

Table. 3: Antibacterial activity of alcohol fraction of *Cadaba fruticosa(L.)* against pathogens by Agar well diffusion method.

S.no	Conc of plant extracts in µg/ well	Zone of Inhibition in mm/ Test organisms			
		S.aureus	***Bacillus* sp.**	***E.coli***	***Pseudomonas* sp**
1	400µg	10.2	6.0	8.6	8.8
2	800µg	11.3	6.4	10.0	9.2
3	1200µg	14.2	7.9	12.0	10.4
4	1600µg	17.4	9.1	14.5	12.4
5	Positive control	20.1	20.1	20.1	20.1
6	Negative control	-	-	-	-

Table. 4: Antibacterial activity of chloroform fraction of *Cadaba fruticosa(L.)* against pathogens by Agar well diffusion method.

S.no	Conc of plant extracts in µg/ well	Zone of Inhibition in mm/ Test organisms			
		S.aureus	***Bacillus* sp.**	***E.coli***	***Pseudomonas* sp**
1	400µg	7.2	8.0	9.6	8.0
2	800µg	8.3	9.4	11.0	9.9
3	1200µg	9.2	10.2	12.8	11.2
4	1600µg	10.0	11.9	13.5	13.2
5	Positive control	20.1	20.1	20.1	20.1
6	Negative control	-	-	-	-

Phytochemical profile of *Cadaba fruticosa(L.)* plant stem extracts and fraction were depicted in Table:1. The presence of quinones, flavonoids, tannin, phenolic compound and coumarins were identified in aqueous extracts. Tannin, Phenolic compounds, coumarin and flavonoids were identified in chloroform fraction. Tannins, Alkaloids, Phenol compounds and coumarins were present in alcohol fraction.

Tannin are a group of phenolics that are found in varying amounts in foods and medicinal plants which have been shown to exert anti-allergic, anti-inflammatory activity. (Parekh & Chanda, 2007a). Plants have provided a source of inspiration for novel drug compounds as plant derived medicines have made significant contribution towards human health.

Flavonoids have been referred to as nature's biological response modifiers because of their inherent ability to modify the body's reaction to allergies, virus and carcinogens which has strong experimental evidence. They show anti -allergic, anti-inflammatory, antimicrobial and anticancer activity. Flavonoids are found in methanol, chloroform and acetone extracts of different parts of *K. foetidissima*. Flavonoids were present in aqueous, alcohol and chloroform extracts of *Cadaba fruticosa(L.).* Our results are supported by the findings of Siciliano *et al.*, (2004) who detected and quantified eight flavonoids, three C-glycosyl and five O-glycosyl flavones in roots, leaves, stems and fruits of *Sechium edule* and reported highest amount of total flavonoids in the leaves, followed by roots and finally by stems. Highest content of total flavonoids was quantified in the methanol extracts of leaves of *Cadaba fruticosa(L.).*

Tannins are polyphenols that are obtained from various parts of different plants showed potential antiviral and antibacterial activity and they are used in leather processing industries also (Robbins, 1980). High content of tannin was observed in the leaf of *K. foetidissima*. This may be the reason why most of the people are using medicinal plants for the treatment of different diseases.

Phenols are reported as anti-tumour agents and to exhibit antiviral and antimicrobial activities, hypotensive effects and antioxidant properties (Robbins, 1980). In the current study, all the three extracts of stem of *Cadaba fruticosa(L.)* showed the presence of phenolic content. High degree precipitation of phenols was observed in methanol leaf extracts of *Cadaba fruticosa(L.)*. (Raghavendra *et al.*, 2006). The presence of the phenolic compounds proved that they must have antimicrobial and antifungal effect.

Antibacterial activity of extracts and fractions of stem of *Cadaba fruticosa**(L.)*** were screened against the common pathogens *viz., pseudomonas* sp *S.aureus, Bacillus* sp. and *E.coli* by agar well diffusion method (Table 2). All the pathogens were sensitive to alcohol, chloroform fractions and aqueous extract. As the concentration of the extracts was increasing, the zone of sensitivity was also increasing. Aqueous extract exhibited maximum zone of inhibition (ZOI) against *S. aureus* (16.8mm) followed by *Pseudomonas* Sp. (14.7 mm). *Bacillus* was least sensitive and exerted only 8.3mm Zone of inhibition at 1600µg concentration. In the present investigation, the most susceptible bacterium was *S. aureus* and *P. aeruginosa.*

The alcohol fraction exhibited maximum zone of inhibition against *S. aureus* (17.4mm) followed by *E.coli* (14.5mm). *Pseudomonas* Sp. showed 12.4 mm zone of inhibition. *Bacillus* was least sensitive and exerted only 9.1mm Zone of inhibition (ZOI) at 1600µg concentration. (Table 3). Chloroform fraction was less effective when compared to other two fractions. Maximum zone of inhibition was observed in *E.coli* (13.5mm) and the least effect was observed in S. *aureus* (10.0 mm) at 1600µg concentration. (Table: 4).

Haymanti Saha *et al.*,.,(2015) reported that, Water and methanol extracts of *Cadaba fruticosa* (L) did not show any zone of inhibition against the 3 bacterial strains *Viz., E. coli, P. aeruginosa* and *B. subtilis*, whereas ethanol showed ZOI of 7, 6 and 4 mm respectively against the pathogens. Similar results were observed in the current study in the case of ethanol fraction. Contradictory results were observed in our study where aqueous extract also showed maximum zone of inhibition.

The study on *Cadaba fruticosa*, has yielded promising results in terms of antioxidant capacity, scavenging activity, antimicrobial and anti-inflammatory responses (Subbaiah and Savithramma, 2013; Mythreyi *et al.*, 2009). Three extract forms of *Cadaba fruticosa*, showed high potency against different bacterial strains. *C. fruticosa* is pharmacologically and clinically active (Arokiyaraj *et al.*, 2008). GC-MS assay of *Cadaba fruticosa* revealed the presence of various derivatives of imidazoles showing antifungal activity. Hence, it could be used as antifungal drugs after further standardization. (Haymanti Saha *et al.*, 2015).

Udhaya Lavinya *et al.*, (2014) reported that the methanolic extract of *C. fruticosa* has significant amount of antimicrobial activity against *Streptococcus pyogenes, Escherichia coli, Klebsiella pneumonia, Salmonella typhi* and *Pseudomonas aeruginosa*. The results were compared with cefotaxime which was used as the standard reference antibiotic. The total

phenolic content of methanolic extract of the leaves of *C. fruticosa* was found to be 39.8±1.92 mg GAE/g (dry basis). Phenolic compounds are a large group of plant secondary metabolites contain aromatic ring with one or more hydroxyl groups. They have hydrogen donors which make them as good antioxidants (David *et al. 2009)*. Flavonoids are the major components of phenolic content in plants .The phenolic content of *C. fruticosa* may contribute to its antioxidant and significant antimicrobial activity [Prakash, 2007].

Our results are supported by the reports of Premnath Shenoy & Yoganarasimhan (2009). They reported that, a polyherbal antidiarrhoeal ayurvedic formulation named as Kutajarista prepared by using *Woodfordia fruticosa* (L.) as an ingredient was having profound antimicrobial activity against *Staphylococcus aureus, Proteus vulgaris, Salmonella typhi, Bacillus pumilus, Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Micrococcus luteus* and *Candida albicans*.

Parekh and Chanda (2007b) observed similar results as that of current results. They reported that, ethanol/methanol extracts of *Woodfordia fruticosa* Kurz were more active than aqueous extracts on six bacterial strains belonging to Enterobacteriaceae, *viz*. *Enterobacter aerogenes* ATCC13048, *Escherichia coli* ATCC25922, *Klebsiella pneumoniae* NCIM2719, *Proteus mirabilis* NCIM 2241, *Proteus vulgaris* NCTC8313, and *Salmonella typhimurium* ATCC23564. Hence, this plant may be used further to isolate and evaluate the therapeutic antimicrobials.

Plants are the important source for the development of new, potential chemotherapeutic agents. The first step towards this goal is the screening of *in vitro* antibacterial activity. In current study aqueous, ethanol and chloroform fractions of *Cadaba fruticosa(L.).* showed promising results in *In-vitro* control of *Escherichia coli, Staphylococcus aureus, Pseudomonas auriginosa* and *Bacillus*. Hence *Cadaba fruticosa (L.)* could be focused further to determine the exact active principle and their usage as potential therapeutic drug.

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