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Research Article

EVALUATION OF ANTIMICROBIAL ACTIVITY OF N-HEXANE EXTRACT OF DIFFERENT PARTS OF PUMPKIN (SEED, LEAVES AND PULP)

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ABSTRACT

The present study deals with the antimicrobial activity of different extracts of pumpkin (Cucurbita pepo L.) (Seed, Leaves and pulp) against seven microorganisms: two Gram-positive bacteria, i.e. Staphylococcus aureus, Bacillus cereus; three Gram-negative bacteria, i.e. Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis and two yeasts, i.e. Candida albicans & C. parapsilosis. Results revealed that all extracts (Seed, Leaves and pulp) were very effective against P. aeruginosa while some extracts showed no inhibition against S. aureus at all tested concentration (20, 40 & 80 µg/ml). The maximum inhibition zone at 80 µg/ml concentration of n-hexane pulp extract was 39 ± 0.1 mm against P. aeruginosa. The minimum concentration (MIC) for n-hexane pulp extract was 1.65 µg/ml for P. aeruginosa, leading to a conclusion that the n-hexane pulp extract of pumpkin was found to be the most potent. Thus it can be concluded that n- hexane pulp extract of Cucurbita pepo L. was more potent as compared n- hexane seed and leaves extract of Cucurbita pepo L.

KEYWORDS: *Cucurbita pepo L; n-Hexane extract; Seeds extract; Leaves extract; Pulp extract; Antimicrobial activity.*

INTRODUCTION

Cucurbita pepo belonging to Cucurbitaceae family, commonly known as "Pitakusmandah" in Sanskrit; "Kaddu" in Hindi and "Squash" or "Red guard pumpkin" in English, has been reported to be valuable against a wide variety of diseases as per traditional literature.

Fruits, seeds and leaves are the most used parts, some of its active compounds are still unknown. The seeds contain material killed tap warm when used as dough evict it out with feces, it is save way without side effects while synthesis drags have serious damage.

The popularity of pumpkin (fruits, seeds and leaves) has been gained as it possess various activities like antidiabetic, antihypertensive, antitumor, immunomodulation, antibacterial, antihypercholesterolemia and antiimflammatory [1, 2].

It is also used in treatment of benign prostatic hypertrophy (BPH) [3]. Seeds have been reported to be used as supportive treatment in functional disorder of bladder [4].

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The physical and chemical properties of pumpkin have been reported [5-7].

Pumpkin extracts have been reported to possess various health benefits due to presence of various chemical constituents and various studies have confirmed the anti-microbial activities [8, 9].

Because of various factors like low potency, poor solubility, development of resistant strains drug toxicity and side effects, use of synthetic anti-mycotic drugs readily available in the market has been minimized [10].

Thus development of new anti-microbial agents for the treatment of various microbial infection is of increasing interest for the scientists.

MATERIAL AND METHODS

Plant Material: Pumpkins were collected from farm near Madhi village, Gujarat. Pulp, seed and leaves were separated. Pulp, seeds and leaves were dried at room temperature and avoided exposure to sunlight to prevent the loss active constituents. [11] Thereafter, these were finely ground to powder using a blender and used immediately.

Authentication: The plant material (seeds, leaves and pulp) were authenticated by Anand agricultural University, Anand, Gujarat.

Preparation of Plant Extract: Ten grams each of dried, powdered pumpkin pulp, seed and leaves were placed in three

different 500-ml Erlenmeyer flasks respectively. Subsequently, a 200-ml each of n-hexane (98%) was added in each flask. After addition of solvent, flasks were shaken vigorously. The crude extracts (seeds, Leaves, pulp) were filtered through a 500-ml sintered glass funnel under vacuum with the filtrates evaporated to dryness under reduced pressure at 40-50°C. The resulting extracts were then stored at 4°C until use. Test microorganisms used: Two Gram positive (Staphylococcus aureus & Bacillus cereus) and three Gram-negative (Escherichia coli, Proteus mirabilis & Pseudomonas aeruginosa); and two yeasts (Candida albicans & Candida parapsilosis) were the test microorganisms used in this study.

Anti-Microbial growth inhibition assay:

Anti-fungal activity assay: Agar well diffusion method was used in anti-fungal assay. The fungal isolates were allowed to grow at 25°C until they sporulated. After sporulation, the spores were harvested to a mixture of sterile glycerol and distilled water and the spores were scraped using a sterile glass rod. Using a glass spreader, 100 µl of standardized fungal spore suspension were spread on Sabouraud dextrose agar (SDA). (Standardized inoculums were first adjusted to an OD 600 nm of 0.1 ml before use). Wells were then bored into the agar media using a sterile 5-mm cork borer and then, wells were filled with 50 ml extract. The inoculated agar plates were allowed to stand on the bench for 1 hr to allow for proper extract diffusion into the media. Plates were incubated at 25°C for 7 days and later, observed zones of inhibition were measured and recorded in mm. Culture media with Streptomycin at concentration of 10µg/ml were used as control to which effects of the extracts on fungal isolates were compared.

Anti-bacterial activity: The agar well diffusion method was used according to Irobi et al. (1994). [12] The bacterial isolates were first grown in nutrient broth for 18-24 hr before use and standardized inoculum to 0.5 McFarland Standards (106 cfuml -1). One ml of standardized inoculum was spread on Mueller-Hinton agar; well were then bored into the agar media using a sterile 5-mm cork borer and the well filled with 50 µl extracts. Thereafter, these were allowed to stand at 37°C. The plates were observed for inhibition zones after 24 hr. A control set was maintained with DMSO and Streptomycin. All experiments were conducted in three replicates.

Minimum Inhibitory Concentration (MIC): Determination of the minimum inhibitory concentration (MIC) of all the extracts was carried out by two-fold serial dilutions method as described by Akinpelu et al. (1994) [13] and MIC were read in µg/ml after being incubated at 37°C for 24 hr and at 25°C for up to 72 hr for bacteria and fungi, respectively.

RESULTS AND DISCUSSION

Table No. 1: Antimicrobial activity of Pumpkin (seeds, Leaves and Pulp) extract on seven different microorganisms

EXTRACT	CONC. (µg/ml)	S. aureus	B. cereus	E. coli	P. mirabilis	P. aeruginosa	C. albicans	C. parapsilosis
PULP	20	-	13 ± 0.6	-	15.2±0.7	18±0.1	12.1±0.4	12.2±0.4
	40	-	14 ± 0.7	19.5±0.3	25.3±0.8	21± 0.4	14.2±0.7	14±0.2
	80	12.2 ± 0.5	30 ± 0.1	28±1.0	25.4±0.5	39± 0.1	12.1±0.1	18.1±0.6
SEED	20	-	11.5 ± 0.2	-	13 ± 0.1	16 ± 0.5	10 ± 0.2	10.2 ± 0.4
	40	-	12.20 ± 0.6	18 ± 0.5	22 ± 0.5	19.8 ± 0.2	13.5 ± 0.6	13. 60 ± 0.1
	80	11.5 ± 0.1	28 ± 0.6	25 ± 0.1	22.5 ± 0.3	35 ± 0.8	13 ± 0.5	12.8 ± 0.5
LEAVES	20	-	11.8 ± 0.5	-	12.18 ± 0.2	15.8 ± 0.2	10.12 ± 0.8	10.5 ± 0.5
	40	-	12 ± 0.1	17.12 ± 0.6	21.10 ± 0.7	19 ± 0.2	12.10 ± 1.0	12 ± 0.8
	80	12.5 ± 0.4	27.5 ± 0.5	23.90 ± 0.2	11.8 ± 0.5	34.5 ± 0.8	12.5 ± 0.8	12.20 ± 0.1

Table No. 2: Extracts (seeds, leaves, Pulp) minimum inhibitory concentrations of test microorganisms

EXTRACT	S. aureus	B. cereus	E. coli	P. mirabilis	P. aeruginosa	C. albicans	C. parapsilosis
PULP	6.18	3.15	13.10	12.90	1.65	>25	>25
SEED	6.20	5.12	15.50	14.5	2.50	>25	>25
LEAVES	7.12	6.15	15.80	15.0	2.80	>25	>25

In the present study, the anti-microbial activities of different n-Hexane extracts (Leaves, seed and pulp) of *Cucurbita pepo* were determined. Table 1 shows the inhibition zones observed at different concentrations of n-hexane extracts (Leaves, seed and pulp) of *Cucurbita pepo.*

The pumpkin pulp extracts had abroad spectrum on Gram-positive, Gram-negative bacteria and fungi. This finding is in agreement with the findings of many researchers [14-17].

At the highest concentration of 80 µg/ml of *Cucurbita pepo* pulp extract, the mean maximum and minimum inhibition zone was 39.0 ± 0.4 mm against P. aeruginosa; 12.2 ± 0.5 mm against S. aureus, respectively while there was no effect on S. aureus and E. coli at concentration 20 µg/ml.

Cucurbita pepo seed and leaves extract were found to be less effective as compared to *Cucurbita pepo* pulp extract.

For *Cucurbita pepo* seed extract, at the highest concentration of 80 µg/ml, the mean of maximum inhibition zone was 35 ± 0.8 mm against P. aeruginosa and mean of minimum inhibition zone was 11.5 ± 0.1 mm against S. aureus. While there was no effect on S. aureus and E. coli at concentration 20 µg/ml.

For *Cucurbita pepo* Leaves extract, at the highest concentration of 80 µg/ml, the mean of maximum inhibition zone was 34.5 ± 0.8 mm against P. aeruginosa and mean of minimum inhibition zone was 12.5 ± 0.4 mm against S. aureus. While there was no effect on S. aureus and E. coli at concentration 20 µg/ml.

The results of this study agrees previously reported studies of pumpkin extracts possessing anti-microbial activity against Gram-positive bacteria and fungi [7, 18, 19].

It had been reported that the methanolic extract of pumpkin had more anti-microbial effect than aqueous extract [20].

Methanol being polar in nature, can be used for extraction of various polar compounds, but certain groups of non-polar compounds show fair solubility in methanol. The molecule of methanol consists of a single atom of a tetrahedral carbon linked to 3 hydrogen and a -OH group. The -OH group is the polar group and the three hydrogen molecules are the water insoluble hydrocarbon chain. That is why methanol can dissolve polar molecules and non-polar compounds can be extracted with n-hexane.

Pumpkin has good beta-carotene, carbohydrates, vitamins, minerals, amino acids and polysaccharides which including bound protein are the bioactive materials of pumpkin [21-23].

Gram-bacteria have lipopolysaccharides in their cell wall which may prevent the active compounds from reaching the cytoplasmic membrane of bacteria. Generally, the overall impact of pumpkin extracts against the seven microorganisms was clearly shown where the effects of extracts increase with the increase of extract concentrations. The n-hexane extract of pumpkin pulp was found to be the best of other extracts.

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