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Research Article

Antibacterial and Antifungal Effects of *Carica papaya* and *Cucurbita specie* Seed Extracts on *Escherichia coli* and *Aspergillus flavus*

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ABSTRACT

Objective: In this work, we studied the antifungal and anti-bacterial properties of seeds of *Carica papaya* and *Curcubita specie* using selected bacteria and fungi.

Methods: Modified cold extraction method with ethanol and n-hexane was conducted. Antimicrobial properties of the extracts were done using agar block dilution for fungi and agar diffusion method for the bacteria. Measurement of the mean growth rate (MGR) for the fungi isolate and the inhibition zone diameter (IZD) for the bacteria were used as parameters.

Results: Significant antifungal property was observed in ethanolic extract of *Carica papaya* at a concentration of 6% at four days of its exposure, while n-hexane extract of *Carica papaya* and ethanolic extract of *Curcubita specie* show fungistatic action. Ethanolic extract of *Carica papaya* at 6% concentration showed more antifungal property than the control drug. Antibacterial action for all the test extracts was poor, with the control drug showing more significant action than the extracts. There was a statistical significance difference between the ethanolic extract of *Carica papaya* and *Curcubita specie* ($p < 0.05$).

Conclusion: This is an indication that ethanolic extract of *Carica papaya* can be used in the treatment of some of the fungal infection caused by *Aspergillus flavus* likewise n-hexane extracts.

Keywords: Antifungal, Anti-bacterial, *Carica papaya*, *Curcubita specie*, ethanolic extract, n-hexane extract.

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INTRODUCTION

Microbes are tiny forms of life that surround us and they are ubiquitous. The human body is also home to millions of these microbes. In the human body, these microbes can exist either as normal flora, when they occur as commensal or they may cause disease conditions in which case they are pathogenic. The most common of all the microbes are bacteria, fungi and virus; there are also microbes from groups of protozoa. When these microbes constitute sources and factors of disease processes for humans and animals, need arises to ensure control and treatment of diseases caused by these microorganisms, in other to create a balance in the ecosystem. Chemotherapy has been the norm ever since the

discovery of penicillin, but further awareness has been made concerning the possibility involved in the use of plant extracts which are readily available for these purposes. This situation now leads to the coining of the word medicinal plant. A medicinal plant is any plant with one or more of its organs containing phytochemicals that can be used for therapeutic purpose or which are precursor for synthesis of useful drugs¹. The medicinal value of some plants lies in some chemical substance that produce definite physiological actions on the human body²; *Carica papaya* (pawpaw) and *Cucurbita specie* (pumpkin) are part of these medicinal plants.

Medicinal plants are important elements of indigenous medicinal system all over the world. The ethno-botany provides a rich resource for natural drug research and development³. Natural products have played an important role throughout the world in the control, treatment and prevention of human disease. Some authors like Jaiswal *et al.*,⁴ and Akinyemi *et al.*,² reported that the use of medicinal plant throughout the world predated the introduction of antibiotics and others modern drugs. *Carica papaya* is a member of the *Caricaceae* plants and dicotyledonous polygamous diploid specie⁵. It originated from Southern Mexico, Central America and northern part of South America. It is now cultivated in many tropical countries like Indonesia, India and in West Africa. The plant is a perennial plant recognized by its' weak and usually unbranched soft stem, yielding copious latex and crowded by a terminal cluster of large and long stalked leaves. It is readily growing and can grow up to ten meters tall⁶. Literature documented that the phytochemical analysis of the plant showed the presence of many biological important active compounds such as chemopapain, papain, caricain, glycyloendopeptidase and papaya lipase^{7, 8}. Antibacterial screening of seed extracts showed the presence of alkaloids, glycosides as shown by formation of precipitate⁹. The presence of isothiocyanatomethyl, thiocyanic acid and 1,2,4 trimethyl in *Carica papaya* seed extracts may be the reason for its antifungal activities especially against *A.flavus*. Antibacterial activities was also noted due to the presence of its' anti-methylbenzene and 4-methyl thiobutylisocyanate which have antibacterial activities especially against *Psuedomonas pesti* and *E.coli*¹⁰.

Curcubita specie is an angiosperm belonging to the cucurbitaceae family. It is popularly called pumpkin. It is a perennial creeping herb of the gourd family *Cucurbitaceae*. It can be up to ten meters long, hirsute stem, grooved cordate and lobed leaves. Its origin is uncertain as it has been cultivated for a long time, that the wild form no longer exist. The term pumpkin refers to a variety of *Curcubita specie* like *C. pepo*, *C. monchata Dutch*, *C. mixta pang*, *C. maxima Dutch*. The gourd family includes melon, cucumber, squash and gourds (Figure 1) Pumpkins are reported to have medicinal properties. *C.moschata* was reported to have antioxidant components including vitamins A and E, carotene, xanthophylls and phenolic compounds. These have the principal role in protecting against oxidative tissue damage¹¹.

The mechanism of action of different antibiotics varies depending on the composition. Therefore, this research work was embarked upon to improve on these trends, by providing extracts of *Carica papaya* and *Curcubita specie* with effective anti-bacterial and anti-fungal activities, and with the aim of comparing the effectiveness of extracts of *Carica papaya* and *Curcubita specie* against bacteria and fungi

MATERIALS AND METHODS

Study area

Some parts of Enugu metropolis were chosen for the study area where access to many *Caricapapaya* and *Curcubita specie* were available.

Study period

This study was done between May 2019 and February 2020 in Enugu and its environs.

Collection and Identification of seeds

Fresh fruits of *Carica papaya* and *Curcubita specie* identified by the botanist in the department of botany and

biotechnology, University of Nigeria, were bought from some local markets, in Enugu State, Nigeria.

Preparation of the seeds

The fruits were washed separately in three changes of quantifiable clean water containing 1.0% sodium hypochlorite. The seeds were aseptically collected through fine incision made on the fruits with a fine new scalpel blade. The seeds were rinsed in quantifiable clean water, sieved to remove other fruit parts. The seeds were dried in an oven at 60°C separately for 7days and packaged in an airtight container. They were grinded separately using manual grinder until fine homogeneous powders were produced. The powder obtained were packaged in two separate bags and kept in a cool and dry place until when needed for extraction.

Extraction

A modified Cold extraction method according to Kalia and Rashmi¹² was performed, whereby 450grams of the powdered seeds of *Carica papaya* and *Curcubita specie* were separately placed in a large bowl containing 2.5 liters of absolute ethanol and same volume of n-hexane. They were placed in a shaking bath at a constant speed of 220rpm with a retention time of 30 minutes. The shaking maceration process was done at room temperature. The crude extract was allowed to evaporate and the extract stored in concentrated form in the solvents.

Media preparation.

Sabouraud dextrose agar (SDA), nutrient agar, blood agar and MacConkey agar were prepared according to manufacturer instructions.

Bacterial and fungal cultures.

Bacteria and mycological samples were collected from stock culture at laboratory in the Department of medical laboratory sciences, university of Nigeria Enugu campus. Fungal growth was allowed for two weeks with the test tube sealed with cotton wool. The isolate was maintained on 2% Potato Dextrose Agar (PDA) agar medium for 7 days at 28 ± 2°C, with addition of 0.05g/L chloramphenicol as antibacterial agent, with minor modification. The isolate was identified microscopically. Bacteria samples were inoculated into nutrient agar and MacConkey agar. The plates were sealed with masking tape and stored in an incubator at 37°C for 24 hours. Gram staining, biochemical test include indole, methyl red, Vogesprouskar, citrate tests and lacto-phenol cotton blue stain were done according to standard microbial confirmation techniques¹³, for confirmation of the bacteria and fungi used in this study respectively.

Preparation of bacterial test medium:

Nutrient agar, blood agar and Mac-conkey agar media were prepared using standard bacteriological methods of media preparation according Chessbough¹³ and Mohit *et al.*,¹⁴ using agar well diffusion method. The sterilized media were poured into four petri dishes for each media and were allowed to cool and solidify, three from each media served for test sets and one served as a positive control. Three 5mm wells were dug into each of the tests medium in each petri dish and each well was labeled behind according to the extract to be placed into it. Three drops of each of the extracts of n-hexane *Carica papaya* seed extract, ethanolic *Carica papaya* seed extract and ethanolic *Curcubita specie* seed extract from a pipette were added into each of the well as labeled, after inoculation of the bacterium (*E.coli*). This was done for each of the three petri dishes for each media. The control set for each medium had three wells in it, with

three drops of 625mg of Augmentin dissolved in 5ml of sterile water in a test tube for each well as control. After inoculation of the bacterium (*E.coli*) and they were labeled. All plates were covered and allowed to incubate for 24 hours at 37°C inside an incubator. The clear zone around the well was measured in millimeter and defined as inhibition zone diameter (IZD) of the test bacteria.

Inoculation of the agar block:

According to Mohit, *et al.*¹⁴ using agar dilution method, 5mm of agar block containing the fungal organism was embedded on SDA containing the n-hexane and ethanolic extracts of *Carica papaya* and *Curcubita specie* in concentrations of 2%, 4% and 6% to 20ml column of the medium separately. The control set was a fixed concentration of 50mg fluconazole in 6ml of injection water. A negative control of the same volume of SDA medium with nothing added to it was prepared except the agar block. Growth inhibition measurements were taken for 5 days.

Statistical analysis

All data generated were subjected to statistical analysis using two way ANOVA.

RESULTS AND DISCUSSION

Plant extracts have been used as sources of medicine over the years; they constitute sources of indigenous medicine¹⁵. Usually they are either taken whole or their constituent extracted. Most of these extractions have been done by the use of water or other polar solvents¹⁴. *Carica papaya* and *Curcubita specie* are some of the plants that have been used for these purposes. *Carica papaya* is a perennial herbaceous succulent plant with self supporting stem, usually found in the tropics of many parts of the world¹⁶. It has been used for the treatment of indigestion, diarrhoea, blindness, ringworm and stoppage of urination¹⁷. It contains many active agents like papain, chymopapain, benzylglusinate, trypsin, flavinoid and caricin. Its action on organism may be as a result of the lytic enzymes found in the plant parts which act on target sites on organisms, this may be its' possible mode of action. *Curcubita specie* generally called pumpkins is perennial creeping plants of the gourd family. It is an angiosperm belonging to the *Curcubiteaceae* family. Its nutritional components include: polysaccharides, active proteins, carotenoids and minerals. Considerable attentions have been focused on this plant because of its health benefits¹⁸. Scientists found out that its biological activities ranges from antimicrobial to antitumor actions¹⁹.

The seed of *Curcubita* have pharmacological activities such as antifungal, antibacterial and anti-inflammatory activities²⁰. Fungi and bacteria are the most common microbes which are agents of considerable health challenge to man, they have been implicated in disease conditions involving both plants and animals. They are ubiquitous and have been the subject of many researches in the quest to find suitable mode of control and elimination of disease condition caused by these organisms. In this work, we investigated the antibacterial and antifungal properties of the seed extracts of *Carica papaya* and *Curcubita specie* using *E. coli*. and *A. flavus* as the bacterial and fungal test organisms respectively. The arrangement of the inhibition zone diameter (IZD) of the standard agar blocks were shown in Table 1. Growth from 5mm agar block of the test organism was measured according to Mohit *et al.*¹⁴.

Table 2 shows the result for the growth measurement of *A. flavus* in different extracts of *C. papaya* and *Curcubita specie*. The result showed that ethanolic extracts is more effective in fungicidal action as the extract concentration increases. The

reason could be due the fact that papain, which is one of the constituents of *Carica papaya*, has a good antioxidant effect that is not affected by this extracting solvent on day 2²¹. This implies that it's more effective to superficial mycoses than deep-seated mycoses whereas n-hexane extract of *C.papaya* and ethanolic extracts of *Curcubita specie* had neither fungicidal nor fungistatic action shown by reduction in the length or size of standard agar block (5mm), hence there was no statistical significant difference among them (P > 0.05). This could be due to the fact that the extracts had not been properly absorbed by the fungal organism as a result of components of fungal cell walls which offer resistance and protection to the fungi cell, thereby reducing absorption and other possible external mechanical effects from the elements²². Hence no effects were observed. This disagrees with the work done by Consolacion and Kathleen²³, who worked on Sterols from *Curcubita maxima* on study of flowers afforded a 4:1 mixture of spinasterol and 24-ethyl-5a-cholesta-7, 22, 25-trien-3β-ol. Their results showed slight activity against fungi *A. niger* and *C. albicans*; and bacteria *B. subtilis* and *P. aeruginosa*. It disagreed with the work done by Neelamma *et al.*²⁴ in Tamil Nadu, India on phytochemical and pharmacological overview of *Curcubita maxima* and future perspective as potential phytotherapeutic agent. In the study, ethanol seed extract showed a spectrum of inhibition on *Staphylococcus aureus*, *B. subtilis*, *P. mirabilis*, *K. pneumonia* and *E. coli*. The differences in the results obtained could be due to geographical seasonalities.

Table 3 shows the growth measurement of *A. flavus* in ethanolic and n-hexane extracts of *C. papaya* and *Curcubita specie* on day three. The least level of inhibition was shown with 2% n-hexane extract of *C. papaya* (5.0mm) while the highest level of inhibition was shown with 6% concentration of ethanolic extract of *C. papaya* (5.7mm). Tables 4 and 5 showed that as there was increase in concentrations of the extracts with increase in the number of days, there was decrease in mean growth measurements of *A.flavus*. At concentration of 6% there was highest inhibitory level, with ethanolic extract of *C. papaya* showing complete fungicidal action on days four and five; hence there was a statistical significant difference among all the concentrations for all the results of different extracts (P < 0.05). The 2% concentration showed the least level of inhibition, with similar result in the negative control on days four and five for extracts of *C. papaya* in n-hexane and ethanolic extract of *Curcubita*, otherwise there was no statistical significant difference among them when the result was compared with the negative control (P > 0.05). Chukwuemeka and Anthonia²⁵ reported similar result although with *Aspergillus niger*, in which higher inhibition was reported in higher concentration of semi riped *C. papaya* seed extract from Ogun state, Nigeria. Furthermore, ethanolic extract of *Curcubita* showed the least inhibitory action in the mean growth of *A. flavus*. At concentration of 4%, *Curcubita* extract have less fungistatic and bacteriostatic actions than 4% of ethanolic extract of *Carica papaya*, with 18mm measured for *Carica papaya* in day 4 and 48mm for *Curcubita* extract for day 5. This may be due to difference in biological constituent of lytic enzyme in *C. papaya* with higher degree of solubility in ethanol than *Curcubita specie*²⁵. This result disagrees with work done by Amna²⁶ in Saudi Arabia, who reported antifungal action in *Curcubita specie*, using aqueous extract, and highest level of inhibition was seen at 2% of the extract after 6 days. This shows that difference in solubility of the content of the seed extract in different solvent either polar or non-polar may be responsible for the action of the extract on the test organism. Similar result was also reported by Wang and Ng²⁰, who reported antifungal properties from *Curcubita moschata* seed extract.

Figures 1 to 3 showed the inhibitory effects of the various extracts at varying concentrations (2%, 4% and 6%) on the mean fungal growth from day 1 to day 5. In all the figures, increasing concentration give lower mean growth measurement of *A. flavus*. With increase in length of days, growth of *A. flavus* was observed to decrease in extracts of *Curcubita specie*. At concentration of 2%, n-hexane extract of *C. papaya* and ethanolic extract of *Curcubita* showed little inhibitory effect but at 6% concentration there was

complete inhibition (fungicidal action) on days 4 and 5 for ethanolic extract of *Carica papaya* compared to the negative control plate; hence, this showed a marked statistical significant difference between ethanolic extract of *Carica papaya* and ethanolic extract of *Curcubita specie* ($P < 0.05$). This agrees with the work of Chukwuemeka and Anthonia²⁵, who showed that the antifungal action of *Carica papaya* seed extract could be due to its lytic enzyme action on sugar of the cell wall of the fungi.

Table 1: Mean growth measurement (mm) of *A. flavus* at different concentrations in different extracts on Day one.

Concentration (%)	Ethanolic extract of <i>C.papaya</i> (mm)	n-hexane extract of <i>C.papaya</i> (mm)	Ethanolic extract of <i>Cucurbita specie</i> (mm)
2	5.0	5.0	5.0
4	5.0	5.0	5.0
6	5.0	5.0	5.0
Positive control (50mg Fluconazole)	5.0	5.0	5.0
Negative control	5.0	5.0	5.0

Table 2: Mean growth measurement (mm) of *A.flavus* at different concentration in different extracts on Day two.

Concentration %	Fungi	Ethanolic extract of <i>C. papaya</i> (mm)	n-hexane extract of <i>C. papaya</i> (mm)	Ethanolic extract of <i>Curcubita sp.</i> (mm)
2	<i>A.flavus</i>	5.3	5.7	6.3
4	<i>A.flavus</i>	5.7	5.5	7.5
6	<i>A.flavus</i>	5.0	7.0	5.0
Positive control (50mg Fluconazole)	<i>A.flavus</i>	6.0	6.0	6.0
Negative control	<i>A.flavus</i>	8.5	8.5	8.5

Table 3: Mean growth measurement (mm) of *A.flavus* at different concentration in different extracts on Day three.

Concentration %	Fungi	Ethanolic extract of <i>C. papaya</i> (mm)	n-hexane extract of <i>C.papaya</i> (mm)	Ethanolic extract of <i>Curcubita sp</i> (mm)
2	<i>A.flavus</i>	23.5	50.0	42.5
4	<i>A.flavus</i>	4.5	32.3	33.2
6	<i>A.flavus</i>	5.7	33.2	37.2
Positive control (50mg fluconazole)	<i>A. flavus</i>	14.6	14.6	14.6
Negative control	<i>A. flavus</i>	86.0	86.0	86.0

Table 4: Mean growth measurement of *A.flavus* at different concentrations in different extracts on Day four.

Concentration %	Fungi	Ethanolic extract of <i>C. papaya</i> (mm).	n-hexane extract of <i>C. papaya</i> (mm).	Ethanolic extract of <i>Curcubita specie</i> (mm)
2	<i>A.flavus</i>	33.5	86.0	86.0
4	<i>A.flavus</i>	18.0	33.5	53.2
6	<i>A.flavus</i>	-	41.2	51.0
Positive control (50mg Fluconazole)	<i>A. flavus</i>	14.6	14.6	14.6
Negative control	<i>A.flavus</i>	86.0	86.0	86.0

Table 5: Mean growth measurement (mm) of *A. flavus* at different concentration in different extracts on Day five.

Concentration %	Fungi	Ethanollic extract of <i>C. papaya</i> (mm).	n-hexane extract of <i>C. papaya</i> (mm).	Ethanollic extract of <i>Curcubitasp</i> (mm)
2	<i>A.flavus</i>	37.5	86.0	86.0
4	<i>A.flavus</i>	26.5	51.5	60.2
6	<i>A.flavus</i>	-	40.0	48.0
Positive control (50mg Fluconazole)	<i>A.flavus</i>	17.0	21.2	10.2
Negative control	<i>A.flavus</i>	86.0	86.0	86.0



Fig 1a: *C. pepo* pumpkins: two bright orange ones in center right, and squashes *C. Maxima*: all others



Figure 1b: Cucurbita; Squash; Pumpkin: seeds



Fig 1c: Fruits and seeds of *Carica papay*

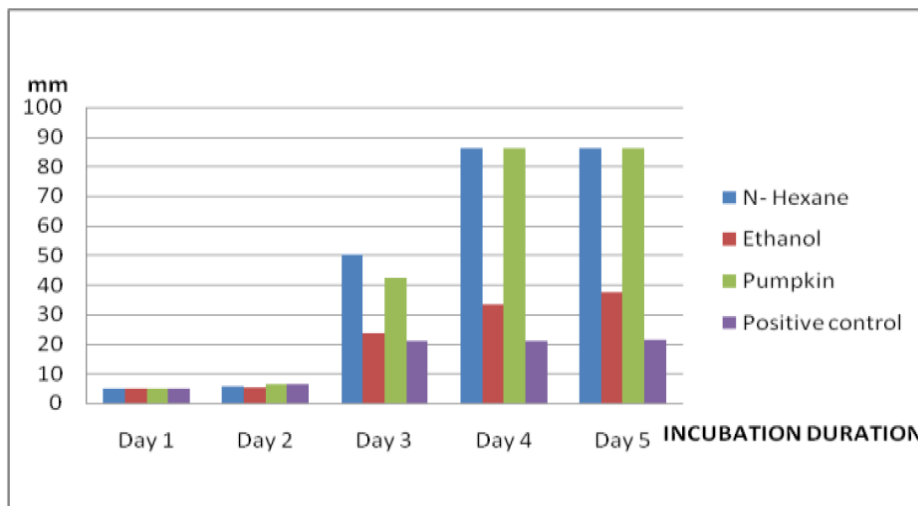


Figure 2: Mean growth measurement (mm) of *A. flavus* at 2% concentration in different extracts of *C. papaya* and *Cucurbita specie* from day 1-5.

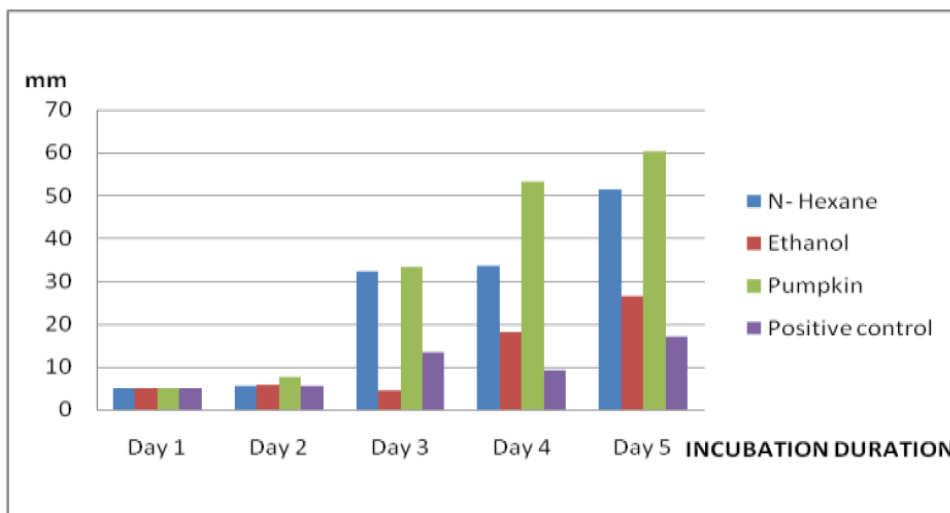


Figure 3: Mean growth measurement (mm) of *A. flavus* at 4% concentration in different extracts of *C.papaya* and *Cucurbita specie* from day 1-5.

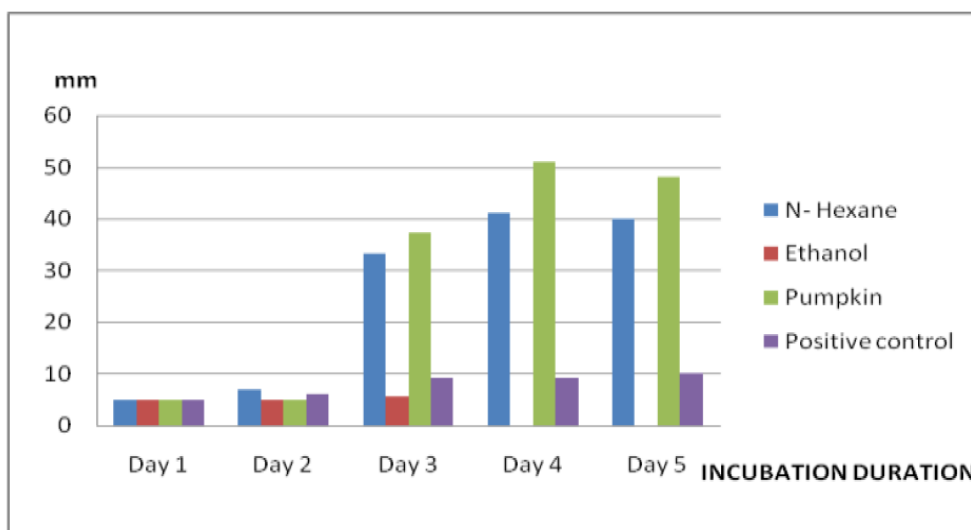


Figure 4: Mean growth measurement (mm) of *A.flavus* at 6% concentration in different extracts of *C.papaya* and *Cucurbita specie* from day 1-5.

CONCLUSION

It was observed that ethanolic extracts of *Carica papaya* seeds were more effective as anti-fungal and antibacterial agents than n-hexane extracts of *Carica papaya* seeds and ethanolic extracts of *Curcubita specie seeds*. However, low antibacterial action was noted in all the extracts against the test organism (*E.coli*).

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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None.

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