https://doi.org/10.33472/AFJBS.6.9.2024.3026-3034

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Unveiling the remarkable antimicrobial, antioxidant, and cytotoxic properties of Helianthus annuus, and Cucurbita moschata- An in vitro study

[1]Dr. Visalachi MR, [2]Dr. Uma Maheswari T.N

Post Graduate,Department of Oral Medicine and Radiology,
Saveetha Dental College and Hospitals ,Saveetha Institute of Medical and technical Sciences
Saveetha University,Cheenai-600077
Email Id- 152110004.sdc@saveetha.com
Professor and Head of Academics,Department of Oral Medicine and Radiology
Saveetha Dental College and Hospitals ,Saveetha Institute of Medical and technical Sciences
Saveetha University,Cheenai-600077
Email Id- umamaheswaritn@saveetha.com
Corresponding Author: Dr. Uma Maheswari T.N

Volume 6, Issue 9, May 2024

Received: 19 March 2024

Accepted: 12April 2024

Published: 22 May 2024

doi:10.33472/AFJBS.6.9.2024.3026-3034

Abstract:
Introduction:
The common sunflower (Helianthus annuus) and pumpkin (Cucurbita moschata) seeds are valued for their nutritional and medicinal benefits. Sunflower seeds are renowned for their high oil content, while pumpkin seeds, often considered agricultural byproducts, are rich in nutrients and nutraceutical properties. Both seeds contain essential compounds such as phenolic compounds, flavonoids, polyunsaturated fatty acids, and vitamins, offering diverse health benefits including antioxidant, antimicrobial, anti-inflammatory, and wound-healing properties. Additionally, they provide essential minerals and exhibit potential in combating non-communicable disorders and promoting overall health.
The study aimed to assess the antimicrobial, antioxidant, and cytotoxic properties of Helianthus annuus and Cucurbita moschata extracts. Results indicated strong antibacterial efficacy, higher than antifungal activity, along with comparable antioxidant and cytotoxic properties in both plant extracts.
Material and Methods:
Sunflower and pumpkin seeds were processed to extract bioactive compounds for antimicrobial, antioxidant, and cytotoxic property evaluations. After meticulous cleaning and drying, the seeds were ground into a fine powder and dissolved in distilled water. The resulting solution underwent filtration and refrigeration, followed by controlled heating to evaporate the solvent. Extracts were then prepared at three concentrations.
For antimicrobial assessment, freeze-dried Candida albicans and Streptococcus mutans were reconstituted and cultured with the extracts. Antioxidant activity was measured using a radical scavenging assay with different concentrations of mixed extracts. Cytotoxicity was evaluated on gingival fibroblasts using MTT assay.
Result:
The study found that both 1:3 and 3:1 concentrations of Helianthus annuus and Cucurbita moschata exhibit comparable antioxidant and cytotoxic properties. Both concentrations of the plants showed comparable antioxidant properties, with outcomes of 86% and 89% for the 3:1 and 1:3 concentrations, respectively. Furthermore, both concentrations exhibited 126% cell viability in cytotoxicity assays. Overall, Helianthus annuus and Cucurbita moschata demonstrate similar levels of antioxidant and cytotoxic properties, suggesting their potential as sources of antimicrobial, antioxidant, and cytotoxic agents.
Conclusion:
The potential influence of diverse seed variants on the study outcomes emphasizes the importance of exploring various varieties. Subsequent large-scale research, including clinical trials are required.

Keywords: Sunflower seeds, pumpkin seeds, antimicrobial, antioxidant, cytotoxic, nutraceutical

Introduction:

The common sunflower (Helianthus annuus) belongs to the Asteraceae family and is cultivated globally, providing a range of nutritional and medicinal advantages. While sunflower seeds are commonly consumed as snacks, used as salad toppings, and incorporated into various baked goods, their main purpose is oil extraction. Sunflower oil holds the fourth position in global oil production, comprising 8% of the total 186 million metric tons in 2012, following palm oil (29%), soybean oil (22%), and oilseed rape (13%). [1] Sunflower seeds and their sprouts are rich sources of essential compounds like phenolic compounds, flavonoids, polyunsaturated fatty acids, and vitamins, which offer a range of health benefits. [2] These include potent antioxidant, antimicrobial, anti-inflammatory, antihypertensive, and wound-healing properties, as well as positive effects on cardiovascular health. Notably, sunflower seeds comprise roughly 20% protein, with their storage proteins supplying valuable sulfur and nitrogen. [3] These sulfur-enriched proteins play a crucial role in various human metabolic processes, such as the development of muscular and skeletal cells, insulin production, and acting as antioxidants. [4] Tocopherols and L-ascorbic acid contribute to the antioxidant effects, along with enzymes like catalase, glutathione dehydrogenase, guaiacol peroxidase, and glutathione reductase, as well as carotenoids. Tannins, saponins, glycosides, alkaloids, and various phenolic compounds exhibit antimicrobial activity. Compounds such as chlorogenic acid, glycosides, phytosterols, caffeic acid, and quinic acid contribute to the antidiabetic effects. 11S globulin peptides are responsible for the antihypertensive effects. α-tocopherol, triterpene glycosides, and helianthosides are involved in anti-inflammatory activity. Linoleic acid and arachidonic acid play a significant role in wound healing. [5]

Pumpkins, scientifically classified under the family Cucurbitaceae, are commonly cultivated as a vegetable in various regions around the world. They thrive in tropical and sub-tropical climates, alongside other related crops like cucumbers and squash. There are three main types of pumpkins globally: Cucurbita pepo, Cucurbita maxima, and Cucurbita moschata. [6] Despite pumpkin seeds being traditionally considered agricultural byproducts, they are actually rich repositories of nutrients and possess noteworthy nutraceutical properties. [7] The consumption of pumpkin seeds is on the rise in numerous African countries and beyond, owing to their recognized health benefits in traditional medicine practices. [8] Frequently, these seeds are enjoyed as snacks after being roasted and seasoned. Additionally, they find applications as food supplements in the baking industry. [9] Plants serve as natural reservoirs of bioactive compounds and are commonly harnessed for their role as functional food ingredients. Pumpkin seeds, much like other seeds, are abundant in a diverse range of functional components.[10] They boast high levels of vitamin E (tocopherols), carotenoids, provitamins, pigments, pyrazine, squalene, saponins, phytosterols, triterpenoids, phenolic compounds, derivatives of phenolics, coumarins, unsaturated fatty acids, flavonoids, and proteins.[11] Furthermore, pumpkin seeds are excellent sources of essential minerals such as magnesium, potassium, and phosphorus, along with trace minerals like zinc, manganese, iron, calcium, sodium, and copper. Some of these bioactive minerals exhibit the potential to act synergistically or individually on various target sites within the body. This capacity makes them beneficial for enhancing overall health and reducing the risk of non-communicable disorders including tumors, microbial infections, hyperglycemia, and diabetes, as well as complications related to oxidative stress, prostate issues, and urinary bladder problems.[12] In addition to these health benefits, pumpkin seed extract (PSE) has demonstrated therapeutic properties such as hepatoprotective effects, stimulation of wound healing and hair growth, anthelmintic (anti-parasitic) activity, antioxidant capabilities, and chemoprotective properties.[13] The aim of the present study was to evaluate the antimicrobial, antioxidant, and cytotoxic properties of *Helianthus annuus* and *Cucurbita moschata*.

Material and methods:

Sunflower and pumpkin seeds were meticulously cleaned, followed by a process of thorough washing and sun-drying. Next, the samples underwent grinding to achieve a finely powdered consistency using a mechanical grinder. Subsequently, 20 grams of each powdered sample were dissolved in 100 ml of distilled water. This mixture was then meticulously filtered through a Whatman NO.1 filter paper, and the resulting solution was collected in a 250ml glass flask. The flask was securely plugged with cotton and stored in a refrigerator at 4˚ C for a period of 24 hours. After this refrigeration period, the contents were filtered once again and then subjected to a controlled heating process in a hot oven, set at 30˚ C. This heating continued for a span of 5 days, ensuring complete evaporation of the solvent. Following this, three distinct concentrations of each extract were prepared by diluting them with ethanol (fig-1,2).

Anti-Microbial Property:

Freeze-dried *Candida albicans(C.albicans)* were acquired and then reconstituted by opening the ampules containing the freeze-dried microorganisms and mixing them with distilled water. This mixture was thoroughly stirred using a sterile stirrer and left to stand for thirty minutes. Each prepared mixture was aseptically applied and evenly spread on a sterile culture media plate using a swab. For *C. albicans*, Sabouraud Dextrose Agar (SDA) and brain heart infusion agar for *Streptococcus mutans* (*S.mutans*)were autoclaved at 121°C for 15 minutes. The *C. albicans* samples were inoculated and incubated at 37°C for 2 hours. Subsequently, a cotton streak culture was performed. Five were created, with three for *Helianthus annuus* (*H.annuus*) and *Cucurbita moschata (C.moschata)* extracts at a concentration of 1:3,3:1,1:1, and one for fluconazole, and streptomycin (control group). (Fig-3)

Fig-1 *Helianthus annuus* and *Cucurbita moschata* diluted in ethanol

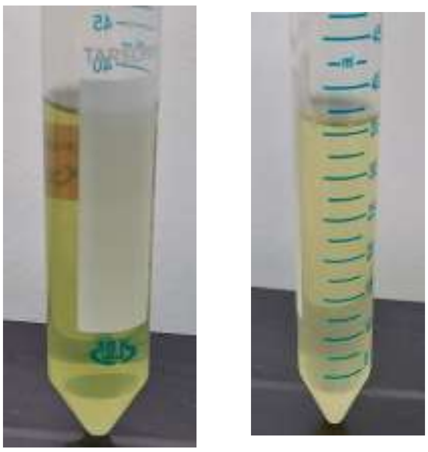


Fig-2 *Helianthus annuus* and *Cucurbita moschata* diluted in ethanol and filtered

Antioxidant Property:

A radical scavenging activity test was conducted at a wavelength of 492 nm using three different concentrations of a mixture composed of *H.annuus* and *C.moschata* in the ratios of 1:1, 3:1, and 1:3, combined with both ascorbic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH).

Cytotoxic Property:

For each of the three concentrations of *H. annuus* and *C. moschata*, 1mL of culture medium containing gingival fibroblasts was added to separate wells in a six-well plate. Following this, 0.5mg/mL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) was introduced to each well. The plate was then gently shaken for two minutes to ensure a uniform mixture. Subsequently, the culture medium was carefully removed, and 100μl of DMSO (Dimethyl sulfoxide) was added to solubilize the crystals. The plates were then incubated at 37°C for four hours. After incubation, 100μl of the DMSO solution was transferred to a 96-well plate, and the absorbance was measured at 450 nm.

Results:

The study revealed that both 1:3 concentration and 3:1 concentration of *Helianthus annuus* and *Cucurbita moschata* exhibit comparable antioxidant and cytotoxic properties. Moreover, the antimicrobial analysis reveals that these plants demonstrate strong antibacterial efficacy in contrast to their antifungal activity. In the 3:1 concentration of *Helianthus annuus* and *Cucurbita moschata*, the zone of inhibition against antibacterial activity measured 17mm. The antioxidant properties of both 3:1 and 1:3 concentrations of *Helianthus annuus* and *Cucurbita moschata* yielded comparable outcomes of 86% and 89%, respectively. (Fig-4 a, b). Additionally, both concentrations of these plants showed 126% cell viability in cytotoxicity assays. The study evaluated the zone of inhibition of *Helianthus annuus* and *Cucurbita moschata* against *Candida albicans (C.albicans), Candida tropicalis (C.tropicalis), Candida parapsilosis (C. parapsilosis)*, and *Streptococcus mutans (S. mutans)*. Results indicate that both plants possess antimicrobial, antioxidant, and cytotoxic properties. Their antibacterial efficacy was notably higher than their antifungal activity. Moreover, both 1:3 and 3:1 concentrations of Helianthus annuus and Cucurbita moschata demonstrated similar outcomes in terms of antioxidant and cytotoxic properties. (Fig 5-a, b, c, d)

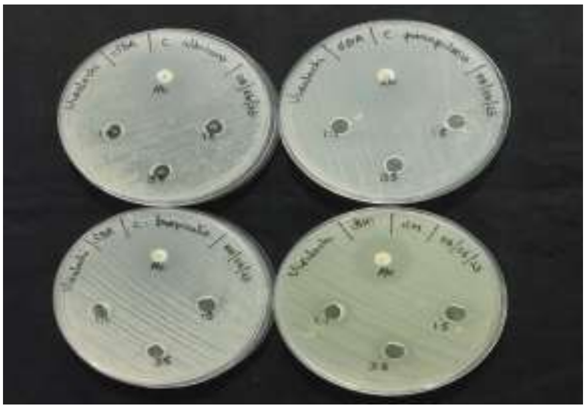
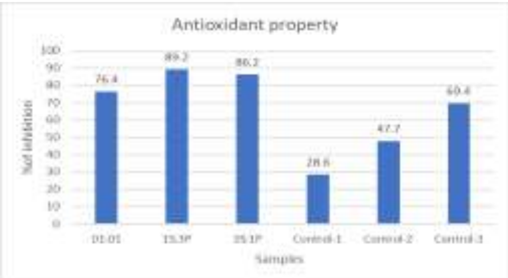
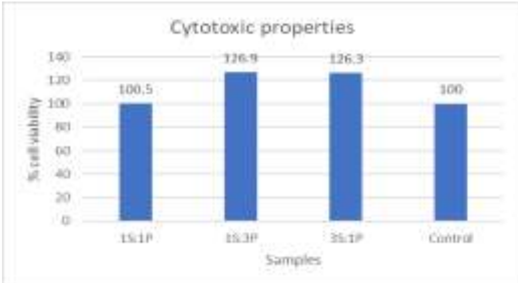


Fig 3: Zone of Inhibition of Helianthus annuus, Cucurbita moschata against Candida albicans, Candida tropicalis, Candida parapsilosis and streptococcus mutans

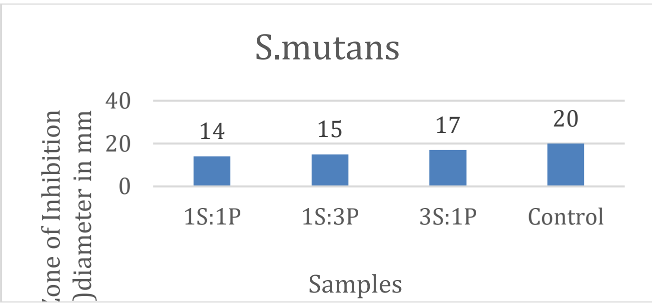


4 a

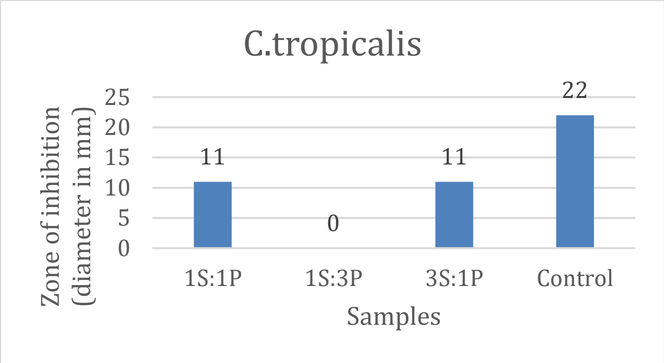


4 b

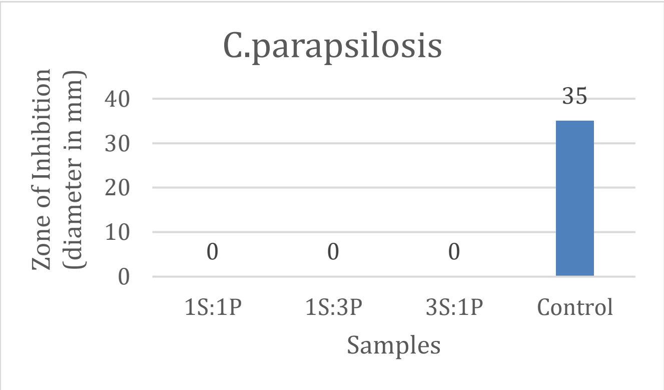
Fig 4 a, b: Antioxidant and cytotoxic properties reveal that both 1:3 conc. &3:1 conc. of *Helianthus annuus*, *Cucurbita moschata* have similar results



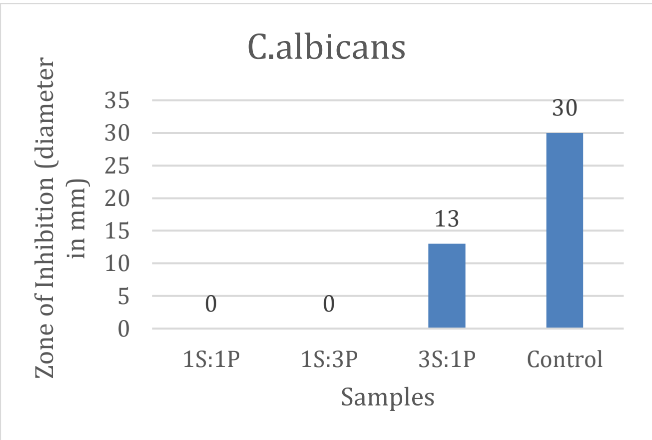
5 a



5 b



5c



5 d

Fig 5 a, b, c, d: Antimicrobial properties of *Helianthus annuus*, *Cucurbita moschata against Candida albicans, Candida tropicalis, Candida parapsilosis,* and *streptococcus mutans reveals Helianthus annuus*, *Cucurbita moschata* have excellent antibacterial property when compared to antifungal. Zone of inhibition for the conc. 3:1 *Helianthus annuus*, *Cucurbita moschata* is 17mm

Discussion:

The current study revealed that both Helianthus annuus and Cucurbita moschata demonstrate similar levels of antioxidant and cytotoxic properties. The findings suggest that both plants possess antimicrobial, antioxidant, and cytotoxic capabilities, with a notably higher effectiveness against bacteria compared to fungi.

Ashiq et al. [14] examined 80% methanol extracts of pumpkin peel, flesh, and seeds powders, noting that pumpkin peel powder yielded the highest extract, while pumpkin seeds powder yielded the lowest. Similarly, Singh et al. [15] conducted extractions of various parts of Cucurbita using different solvents, reporting extract yields of 7.37% for pumpkin peel and 5.43% for pulp, employing 70% methanol extraction. Furthermore, Asif et al. [16] investigated the antioxidant potential of pumpkin peel and puree extracts utilizing the DPPH free radical scavenging assay. They observed promising antioxidant activity in the 99.9% methanolic extracts of pumpkin peel and puree, with values of 68.79% and 69.56%, respectively.

Aboki et al. [17] conducted a study revealing the efficacy of antimicrobial properties in sunflower seeds against various microorganisms including Staphylococcus aureus, Escherichia coli, and Bacillus subtilis. Similarly, findings by Bashir et al. [18] through spectrophotometry analysis of sunflower seeds identified bioactive compounds such as chalcones kukulcan B, helianone A, flavanones heliannones B and C, and flavonol tambulin. Rodhiyah et al [19] research demonstrated that extracts from sunflower seeds expedited wound healing. This capability is attributed to the presence of active compounds such as β-sitosterol, flavonoids, and linoleic acid within the seeds.

Conclusion

Different seed variants could influence the outcome of the study, necessitating experimentation with various varieties. Further research on a larger scale, including clinical trials involving Helianthus annuus and Cucurbita moschata, either in suspension or emulsion form, is necessary.

Reference:

1. Prolea . *De la production à la consommation. Statistiques desOléagineux et Protéagineux.* Paris: Prolea Documentation; 2012. p. 48
2. Fowler MW. Plants, medicines and man. *J Sci Food Agric.* 2006;86(12):1797–1804. doi: 10.1002/jsfa.2598.
3. Youle RJ, Huang AHC. Album in storage proteins in the protein bodies of castor bean. *Plant Physiol.* 1978;61:13–16. doi: 10.1104/pp.61.1.13.
4. Youle RJ, Huang AHC. Occurrence of low molecular weight and high cysteine containing albumin storage proteins in oilseeds of diverse species. *Am J Bot.* 1981;68:44–48. doi: 10.2307/2442990
5. Guo S, Ge Y, Na Jom K. A review of phytochemistry, metabolite changes, and medicinal uses of the common sunflower seed and sprouts (Helianthus annuus L.). Chem Cent J. 2017 Sep 29;11(1):95. doi: 10.1186/s13065-017-0328-7. PMID: 29086881; PMCID: PMC5622016.

6. Lee YK, WI Chung, H Ezura (2003) Efficient Plant Regeneration via Organogenesis in Winter Squash (Cucurbita maxima Duch). Plant Sciences 164 (3): 413-418.
7. M.Z. Amin, T. Islam, M.R. Uddin, M.J. Uddin, M.M. Rahman, M.A. Satter. Comparative study on nutrient contents in the different parts of indigenous and hybrid varieties of pumpkin (Cucurbita maxima Linn.)
8. N. Aktaş, T. Uzlaşır, Y.E. Tunçil. Pre-roasting treatments significantly impact thermal and kinetic characteristics of pumpkin seed oil
9. S.M. Abou-Zeid, H.O. AbuBakr, M.A. Mohamed, A. El-Bahrawy. Ameliorative effect of pumpkin seed oil against emamectin induced toxicity in mice
10. G.G. Adams, S. Imran, S. Wang, A. Mohammad, S. Kok, D.A. Gray, G.A. Channell, G.A. Morris, S.E. Harding. The hypoglycaemic effect of pumpkins as anti-diabetic and functional medicines. Food Res. Int., 44 (4) (2011), pp. 862-867
11. D. Broznić, G. Čanadi Jurešić, Č. Milin. Involvement of α-, γ-and δ-tocopherol isomers from pumpkin (Cucurbita pepo L.) seed oil or oil mixtures in the biphasic DPPH disappearance kinetics. Food Technol. Biotechnol., 54 (2) (2016), pp. 200-210
12. S. Dakeng, S. Duangmano, W. Jiratchariyakul, Y. U-Pratya, O. Bögler, P. Patmasiriwat. Inhibition of Wnt signaling by cucurbitacin B in breast cancer cells: reduction of Wnt-associated proteins and reduced translocation of galectin-3-mediated β-catenin to the nucleus. J. Cell. Biochem., 113 (1) (2012), pp. 49-60
13. K.Y. Chari, P.R. Polu, R.R. Shenoy. An appraisal of pumpkin seed extract in 1, 2-dimethylhydrazine induced colon cancer in wistar rats. J. Toxicol., 2018
14. Hussain, Ashiq & Kausar, Tusneem & Din, Ahmad & Murtaza, Mian & Jamil, Muhammad & Noreen, Saima & Iqbal, Muhammad. (2022). Antioxidant and Antimicrobial Properties of Pumpkin (Cucurbita maxima) Peel, Flesh and Seeds Powders. Journal of Biology, Agriculture and Healthcare. 10.7176/JBAH/11-6-05.
15. Singh, J., Singh, V., Shukla, S., & Rai, A. K. (2016). Phenolic content and antioxidant capacity of selected cucurbit fruits extracted with different solvents. J Nutr Food Sci, 6(565), 2.
16. Asif, M., Raza Naqvi, S. A., Sherazi, T. A., Ahmad, M., Zahoor, A. F., Shahzad, S. A., ... & Mahmood, N. (2017).Antioxidant, antibacterial and antiproliferative activities of pumpkin (cucurbit) peel and puree extracts-an in vitro study. Pakistan journal of pharmaceutical sciences, 30(4), 1327-1334.
17. Aboki, MA et al. 2012. "Physicochemical and Anti-Microbial Properties of Sunflower (Helianthus annuus L.) Seed Oil." International Journal of Science and Technology 2(4): 151–94
18. Bashir, Tasneem et al. 2015. "Chemistry, Pharmacology and Ethnomedicinal Uses of Helianthus annuus (Sunflower): A Review." Pure and Applied Biology 4(2): 226-235.
19. Rodhiyah, and Sulistiyawati. 2012. "Effect of Sunflower (Helianthus annuus) Seed Oil Extract on the Early Process of Wound Healing." Environmental Science Biology 9(1): 706–11.