ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, Dec 2022

Extraction Of Bioactive Compounds From Niger Seed And The Detection Of Their Antimicrobial Activity Against The Pathogenic Microbes

T A Sathya[1*], R. Anburaj[2], Angelina[3]

[1*]Assistant Professor, PG & Research Department of Microbiology, Vivekanandha College of Arts and Sciences (Autonomous) for Women. Elayampalayam, Tiruchencode, Tamilnadu, deepthisha2011@gmail.com

[2]Associate Professor, PG & Research Department of Biotechnology &Microbiology, National College (Autonomous), Tiruchirapalli, Tamilnadu, anbu_nanthu@rediffmail.com

[3]Assistant Professor, Department of B.Voc-Healthcare Technology, St. Philomena's College (Autonomous), Mysore, Karnataka, angelinastanley@stphilos.ac.in

Corresponding Author

Dr T A Sathya M.Sc, M.Phil, Ph. D, DMLT, B.Ed

DST INSPIRE FELLOW

Assistant Professor, PG & Research Department of Microbiology

Vivekanandha College of Arts and Sciences For Women (Autonomous)

Elayampalayam, Tamilnadu.

deepthisha2011@gmail.com

ABSTRACT:

The present study suggests that Indian niger seeds have potential bioactivities. The inherent bioactive molecules may contribute to the stability and radical quenching ability of niger seed oil and cake. The extractability of these bioactive molecules can be increased by the use of polar solvents. Methanol proved to be a good solvent for extraction of both oil and cake and hence revealed the source of antimicrobial activity. Further studies can be made to identify the bioactive principles and also to exploit the products of this widely distributed weed species.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, Dec 2022

KEY WORDS: Niger seeds, Antimicrobial activity, oil extraction, solvent extraction

1. Introduction:

Niger seed (*Guizotia abyssinicia* Cass) is a native of Ethopia. Ethiopia and India are the two major Niger seed producing countries in the world (Ramadan et al 2012). It is widely grown by small and marginalized farmers in India, especially in non irrigated rain fed farms. The global production is estimated between 300,000 and 350,000. India produces an estimated 80000-100000 tonnes with the states of Madhya Pradesh, Andhra Pradesh and Orissa accounting for about 80% of the total population. Niger is a dry season cropping alternative for small holder, upland rice producers in India. It is also recognized as an effective green manure cover crop. Most of the seed is used as bird feed (Lin et al 2005). It is also used in many preparations like chutney powder and also added in curry as flavouring agent. Niger seed oil is used in cooking, burning lamps, making soap and paints. The proximate composition of the niger seed contains about 30% oil and 24% of protein. The oil is rich in oleic oil (26%) and linoleic acid (55%). Due to the high linoleic acid content it has poor storage property. The other fatty acids present in the oil are palmitic acid (8%), stearic acid (8.3%) and arachidic acid (0.5%) (Bhagya et al 2003).

Indian medicinal plants are regularly used in various system of medicine because of minimal side effect and cost effectiveness which provide scientific support to the therapeutic use of the plants in tribal medicine (Rajlakshmi et al 2003). Medicinal plants have been found to be helpful in curing many diseases and have always promoted the search for different extracts from plants which could act as a potential source of new antimicrobial agent (Ahamad et al 1998; Bushra Beegum and Ganga Devi, 2003).

An antimicrobial is a substance that kills or inhibits the growth of microbes such as bacteria, fungi, protozoa or viruses. Antibiotics are those substances which are produced by microorganism that kills or prevents the growth of another microorganism. Antibiotics are generally used against bacteria, antiviral are used specifically for treating viral infections. Several microorganisms derived antibiotics are currently in use to treat a variety of human disease, therefore the action must be taken to control the use of antibiotics, develop new drugs

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, Dec 2022

either synthetic or natural, for a long period of time, plant have a valuable source of natural products for maintaining human health. India has a rich tradition in use of medicinal plants to develop drugs. Screening medicinal plants for biologically active compounds offers clues to develop newer antimicrobial agents. These compounds after possible chemical manipulation provide new and improved drugs to treat the infectious diseases (Natarajan et al 2003, Shah et al 2006). The oil industries give importance to the oil and less attention is given to the utilization of the cake. The protein rich cake is being used as feed due to the presence of high fibre. The oil obtained will be dark in colour. Therefore the seeds were dehulled with a dehulling efficiency of 96-98%. The oil obtained from the dehulled niger seed resukted in light colored oil without affecting the quality of the oil. The oil is nutritious and contains high level of unsaturated fatty acids (oleic and linoleic acids) which reduces blood cholesterol level. The protein is rich in sulphur containing amino acids. The protein has good in vitro digestibility and quality.

The idea behind the work is to improve the oil and cake of niger seeds through dehulling and evaluate the quality and utilization of the cake in other integrated uses also to evaluate the antimicrobial activity. In this regard the value addition to niger seed cake by extraction of bioactive components using different solvents (Methanol, Ethanol, Butanol, Chloroform and Hexane) and check the antimicrobial activity against the microbes (*Salmonella typhimurium, Staphylococcus aureus, Listeria inocua, Pseudomonas aeruginosa and Escherichia coli*) were tested for the bioactivity study.

2. MATERIALS AND METHODS

2.1Bacterial Strains and Maintenance

The bacterial strains *Staphylococcus aureus* FRI 722 (Gram positive), *Salmonella typhimurium* MTCC 1251(Gram Negative), *Escherichia coli* CFR 02 (Gram negative), *Listeria innocua* FB 21(Gram positive) and *Pseudomonas aeruginosa* (Gram negative) were procured from American Type Culture Collection, USA; IMTECH, Chandigarh; ARS Culture Collection (NRRL), USA; and institute culture collection (CFTRI, Mysore). These bacterial strains and the cultures isolated in this study were stored at -20℃ in Brain Heart Infusion (BHI) broth respectively containing 20 % (v/v) glycerol. All the cultures were propagated aerobically twice in LB or BHI broth before

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, Dec 2022

use. For isolating the individual colonies all the strains were streaked on Brain Heart Infusion Agar (BHI) (HI-MEDIA) to obtain pure isolates, following a standard aseptic technique and the four-way streak plate inoculation (Cappuccino and Sherman 2005). The fungal strain (*Aspergillus flavus*) was isolated from the agricultural soil and then inoculated in Potato Dextrose Broth. The colonies were again streaked in the PDA agar plates for getting the individual colony. The stock culture slants were maintained at 4℃.

2.2 Solvents and culture media

BHI broth was prepared and it was sterilized and the solvents used were ethanol, methanol, chloroform, butanol and hexane.

2.3 Collection of Raw material

Niger seeds were obtained from the local market in Karnataka and it was manually separated into hulls and Dehulled were milled prior to extraction. The seeds were cleaned, separated and shade dried for 2 days.

2.4 Solvent Extraction

Five grams of defatted protein rich niger seed cake material i.e both hulled and dehulled (weighed by Shimadzu) was added to 100 mL conical flasks, and 50 mL of each solvent (ethanol, methanol, butanol, hexane and chloroform) was added to each flask. Each mixture was placed on a shaker (Innova New Brunswick Scientific) and left to extract for 48 hours at a speed of about 150 rpm at room temperature (25^{o}C). Extract was then filtered using a conical flask with side arm, a filter funnel (size 2), and a 90 mm diameter filter paper (Whatman #1). Filtered extract was then poured in a weighed 500 mL round bottom flask. Solvent was evaporated under reduced pressure with a rotary evaporator (Buchi, Switzerland). Temperature of the water bath in the rotavapor was set at 40̊C. Evaporation time ranged from 10 to 30 minutes depending on the solvent type. After evaporation of solvent, the flask was weighed again. To determine the weight of the sample, the weight of empty flask was subtracted from the weight of the flask and sample. Concentration was recorded on a weight by volume (w/v) basis.The resulting dried extract was stored in labeled sterile screw-capped bottles at -20 °C. The extract (in the form of sticky black

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, Dec 2022

substances) was dissolved in 1 ml of DMSO before testing. All the extracts were then used for the evaluation for antimicrobial activity.

2.5 Determination of antimicrobial activity

The agar well diffusion method was used to determine the inhibitory effects of the seed extracts against the microbes (Oyeleke et al 2008, Swain et al 1977). The bacterial isolates obtained were first grown in Brain Heart Infusion broth (BHI) for 8-10 hrs at 37℃ and the fungal isolates were first grown in Potato Dextrose Broth at 28-30˚C. Simultaneously, Brain Heart Infusion agar medium (BHI) and the Potato Dextrose Agar (PDA) medium were prepared and an aliquot (0.2 ml) of microbial culture was added to molten BHI and PDA at 45°C and poured into the petriplate. After solidification of the agar, appropriate wells were made on agar surface by using sterile cork borer (5 wells per 90 mm diameter) (Parekh et al 2008). Different concentrations of the extracts were prepared using dimethyl sulfoxide (DMSO) and 50 µl of each concentration was added to the wells. The plates were kept at room temperature for 2 h to allow the extracts to diffuse into the wells. Then the bacterial cultures were incubated at 37°C for 24 hours and the fungal cultures at 30℃ for 24 hours. Antimicrobial activity was determined by measuring the zone of inhibition surrounding the well. The assays were carried out under aseptic conditions. Standard antibiotic Ampicillin, was used as positive control and DMSO as a negative control. The zones of inhibition were measured in millimeter diameter using meter rule (Brindha et al 2009). Each concentration included duplicates and the results are average of two independent experiments.

2.6 Standard plate for the determination of antimicrobial extract

For the preparation of standard plate, the agar well plates were prepared in the same principle of Oyeleke et al 2008. Simultaneously, Brain Heart Infusion agar medium was prepared and an aliquot (0.2 ml) of microbial culture was added to molten BHI at 45°C and poured into the Petriplate. Appropriate wells were made on agar surface by using sterile cork borer (5 wells per 90 mm diameter). 50 µl of each standard extracts of different concentrations were loaded into the appropriate wells. The extract includes the standard melanin with the methanol extract, standard melanin, Methanol extract, Positive control Ampicillin and the negative control DMSO. The antibacterial assay plates were incubated at 37℃ for 24 h.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, Dec 2022

3. RESULTS

The antimicrobial activity of studied species was determined against both gram positive and gram negative bacteria. All the extracts (ethanol, methanol, butanol, hexane and chloroform) showed zone of inhibition against *Salmonella typhimurium*. The zone of inhibition was showed in Fig 3 for both whole and dehulled niger seeds, respectively. The observed zone of inhibition is measured in mm and summarized (Table 1).

Among all the pathogenic organisms tested for the antimicrobial activity *Salmonella thyphimurium* is the only vulnerable organism showed the zone of inhibition against the extracts and also methanolic extracts showed the highest inhibitory activity from both the whole and the dehulled niger seeds. For the comparison of checking the antimicrobial activity with standard against the same organism with the methanolic extracts, standard melanin and the melanin along with the methanolic extracts reveal the same zone of inhibition. The zone of inhibition is showed in Fig 4 for both whole and dehulled niger seeds, respectively in comparison with the positive control and standard melanin. The observed zone of inhibition is measured in mm and summarized (Table 2).

4. DISCUSSION

Antibiotic resistance is a major concern and development of new agents from plants could be useful in meeting the demand for new antimicrobial agents with improved safety and efficacy (Srivastava et al 2000). In this study, it is clearly revealing that the methanol extracts of both whole and dehulled niger seeds exhibited highest antimicrobial activity on *Salmonella typhimurium* compared with the other solvents. It was also found that methanolic extract of *Abrus pulchellus* was more active against Gram-positive bacteria as compared to Gram-negative bacteria. There was further observation that among different solvent extracts of *Solanum seaforthianum* stem, the methanolic extract exhibited high degree of antibacterial activity (Xavier et al 2013). These observations in corroboration with the present finding show that different solvent extracts of a seeds may have different spectra of antimicrobial activity that can be explained by the solubility or insolubility of the active compounds in the solvent used for extraction.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, Dec 2022

5. TABLES

Table 1 Antimicrobial activity of niger seeds

Niger Seeds	Extracts	Pathogenic strains					
		P. aeruginosa	*E.coli*	*S.thphi*	*S.aureus*	*L.inocua*	*A.flavus*
whole							
	Ethanol	-	-	6	-	-	-
	Methanol	-	-	7	-	-	-
	Butanol	-	-	4	-	-	-
	Hexane	-	-	3	-	-	-
	Chloroform	-	-	4	-	-	-
Dehulled							
	Ethanol	-	-	5.6	-	-	-
	Methanol	-	-	7	-	-	-
	Butanol	-	-	5	-	-	-
	Hexane	-	-	3	-	-	-
	Chloroform	-	-	5	-	-	-

Table 2 Comparison of Zone of Inhibition by standard plate

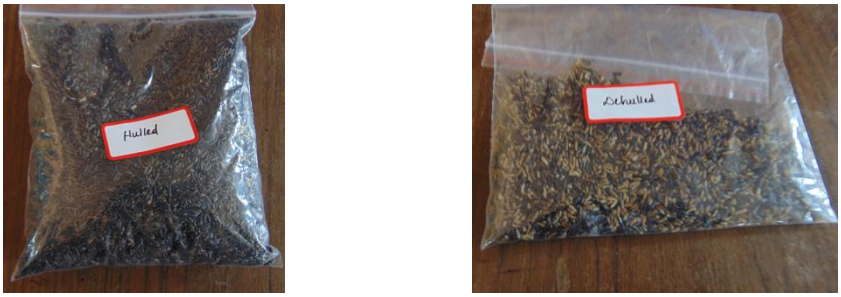
Niger seeds	Methanolic Extracts(mm)	Standard Melanin(mm)	Standard Melanin + Methanolic	Positive control Ampicillin(mm)	Negative control DMSO

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, Dec 2022

			Extracts(mm)		
Whole	4	5	5	11	-
Dehulled	4	6	6	11	-

6. FIGURES



(A) (B)

Fig 1 Niger seeds whole (A) and dehulled (B)

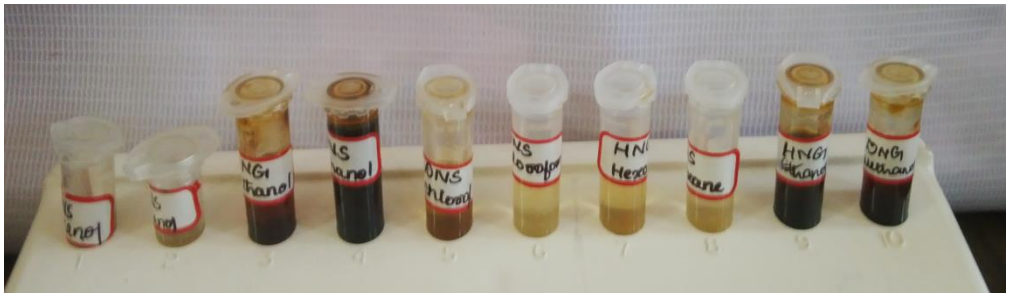
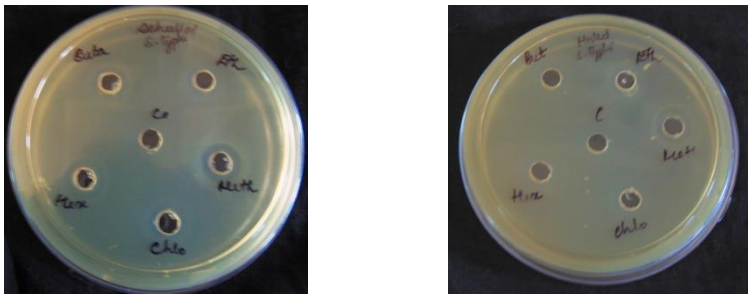


Fig 2 cake dissolved in DMSO after solvent extraction



ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, Dec 2022

(A) **(B)**

Fig 3 Antimicrobial activity of dehulled Niger seeds (A) and whole (B) against *Salmonella typhimurium*

Fig 4 Antimicrobial activity of dehulled Niger seeds (A) and whole (B) against *Salmonella typhimurium* comparison with standard melanin.

.

7. REFERENCES

1. Ahamad I, Mehmood Z, Mohammad F. 1998. *Screening of some Indian medicinal plants for their antimicrobial properties*. J Ethno pharmacol 62: 183-193.
2. Bhagya S, Shamanthaka Sastry MC.2003. *Chemical, functional and nutritional properties of wet dehulled niger (Guizotia abyssinica Cass.) seed flour*. Lebensm.-Wiss. u.-Technol. 36: 703–708.
3. Bhatnagar AS, Gopala Krishna AG.2013. *Effect of Extraction Solvent on Oil and Bioactives Composition of Commercial Indian Niger (Guizotia abyssinica (L.f.) Cass Seed.* J Am Oil Chem Soc 90:1203–1212.
4. Brindha V, Saravanan A and Manimekalai R.2009. *Drug designing for ring finger protein 110 involved in adenocarcinoma (human breast cancer) using casuarin in extracted from Terminalia arjuna.* Indian J Sci Technol 2 (2): 22-26.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, Dec 2022

5. Bushra Beegum NR, Ganga Devi T.2003. *Antibacterial activity of selected seaweeds from Kovalam south west coast of India*. Asian JR of Microbiol Biotech Env Sc 5(3): 319-322.

6. Cappuccino JG, Sherman N. 2005. Microbiology: *A laboratory Manual, 7th Ed*., Pearson Benjamin Community College, New York, NY, p 13-20.

7. Dagne K, Jonsson A.1997. *Oil content and fatty acid composition of seeds of Guizotia* Cass. (Compositae). J Sci Food Agri 73:274–278.

8. Dutta PC, Helmersson S, Kebedu E, Appelqvist LA.1994.*Variation in lipid composition of niger seed (Guizotia abyssinica Cass.) samples collected from different regions in Ethiopia.* J Am Oil Chem Soc 71:839–843.

9. Geleta M, Stymne S, Bryngelsson T.2011. *Variation and inheritance of oil content and fatty acid composition in niger* (*Guizotia abyssinica).* J Food Comp Anal 24:995–1003

10. Gupta C, Garg AP, Uniyal RC, Kumari A. 2008. *Antimicrobial activity of some herbal oils against common food borne pathogens.* Afr J Microbiol Res 5(2):258-261.

11. Marini F, Magrı` AL, Marini D, Balestrieri F (2003*) Characterization of the lipid fraction of Niger seeds (Guizotia abyssinica Cass.) from different regions of Ethiopia and India and chemometric authentication of their geographical origin*. Eur J Lipid Sci Technol 105:697–704

12. Natarajan V, Veugopal P V, Menon T. 2003.*Effect of Azadirachta indica (neem) on the growth pattern of der-matophytes.* Indian J Med Microbiol 21: 98-101.
13. Oyeleke SB, Dauda BEN, Boye OA.2008. *Antibacterial activity of Ficus capensis.* Afr J Biotechnol 7(10): 1414-1417.
14. Parekh J, Chanda S. 2008. *In vitro antifungal activity of methanol extracts of some Indian medicinal plants against pathogenic yeast and moulds*. Afri J Biotechnol 7(23): 4349.

ISSN PRINT 2319 1775 Online 2320 7876

Research paper UGC CARE Listed (Group -I) Journal Volume 11, Iss 12, Dec 2022

15. Rajlakshmi D, Banerjee SK, Sood S, Maulik SK. 2003.*In-vitro and in-vivo antioxidant activity of different extracts of the leaves of Clerodendron colebrookianum Walp in the rat*. J Pharm. Pharmacol 55: 1681-1686.

16. Ramadan MF.2012. *Functional properties, nutritional value and industrial applications of Niger oilseeds* (*Guizotia abyssinica* Cass). Cri Rev Food Sci Nutr 52:1–8

17. Ramadan MF, Moersel JT.2002. *Proximate neutral lipid composition of niger* (*Guizotia abyssinica* Cass.) seed. Czech J Food Sci 20:98–104

18. Ramadan MF, Morsel JT. 2003. *Analysis of glycolipids from black cumin (Nigella sativa L.), coriander (Coriandrum sativum L.) and niger (Guizotia abyssinica Cass.) oilseeds*. Food Chem 80:197–204.

19. Salih HALM, Abass AM. 2003. *Study of the fruit peels of Citrus sinesis* & *Punica granatum*. J Babylon university 3:243-342.

20.Shah JS, Shah MB, Goswami SS, Santani DD. 2006. *Mechanism of action of antiulcer activity of bark extracts of Manilkara hexandra against experimentally induced gastric ulcers in rats.* Phcog Mag 2: 40-45.

21. Srivastava A, Shukla Kumar YN. 2000. *Recent development in plant derived antimicrobial constituents A Review.* J Med Arom Pl Sci 20: 717-72.

22. Swain T. 1977. *Secondary compounds as protective agents*. Annu Rev Plant Physiol 28:479-501.

23.Xavier TF, Kumar PN, Auxillia AS, Kannan M .2013. *In vitro growth inhibition of pathogenic bacteria by Solanum seaforthianum L.* Asian J Plant Sci and Res 3(4): 70-76.