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Invitro Cytotoxicity Assay of Acacia Catechu Ethanolic Seed Extract Using Brine Shrimp

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ABSTRACT

Context: Acacia catechu is a tree which possesses lots of therapeutic value.

Objective: To evaluate the lethality assay of *Acacia catechu* seed using Invitro parameter.

Background: *Acacia catechu* willd is an indigenous plant having tremendous medicinal properties and well reviewed in literature. Ethanolic extract of Acacia catechu sed has already been reported to possess selected pharmacological activities like anti diabetic,but there are no reports on cytotoxicity assay of this extract.

Methods: Twenty nauplii were added into three replicates of each concentration of the plant extract. After 24,48 hours, the surviving brine shrimps were counted and percentage of mortality was assessed

Results: The present study reveals that *Acacia catechu* possess significant cytotoxic activity when tested in vitro.

Conclusion: From the present study it can be concluded that ethanolic seed extract of *Acacia catechu* possessed marked cytotoxic effect The effect was plausibly due to the presence of phytochemical contents present in it.

Clinical significance: Novel cytotoxic, pesticidal compounds can be isolated from plant based sources through the investigation of cytotoxic activity against brine shrimps

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INTRODUCTION

Acacia catechu [1] belongs to the family fabaceae also known as black cutch and karungali in Tamil. In Kerala people drink it in boiling water to prevent digestive problems. Various part of the plant leaves, bark and heartwood possess diverse pharmacological actions for management of various disorders [2,3,4,5]. The pharmacological activities[6,7,8] in various parts of the plant has been extensively studied. Phytochemical constituents like Catechin, Epicatechin, Cyanidol, Quercetin, Epigallocatechin gallate, Rutin, Isorhamnetin, Taxifolin are found to be present in *A.catechu* [9,10]. The various part of the extract reports the antipyretic, anti-inflammatory, antidiarrheal, hypoglycemic, hepatoprotective, antioxidant and antimicrobial activities including anti caries and anti plaque activity.[11,12,13,14,15,16]

MATERIALS AND METHOD

Plant material

Acacia catechu seeds were collected from Hosur, Tamil Nadu and were authenticated by Green Chem lab, Bengaluru, India.

Preparation of ethanolic extract

Seeds were shade dried for a week. Dried seeds were milled to fine powder. Powder was passed through 100 mesh sieve and stored in a sealed polythene bag.2.5kg of powdered *Acacia catechu* seeds were extracted with 10 liters of Ethanol, at 65°C temperature, for 1 hour, in a 20 liter round bottom flask with Graham condenser attached. Condenser was cooled circulating with chilled water. After 1 hour of extraction, the round bottom flask was cooled to room temp and the extract was filtered and collected. The Marc was extracted repeatedly with 10 liters of Ethanol, twice. The extracts were filtered and collected. The combined extracts were evaporated to dryness under reduced pressure in a Buchi Rotary Evaporator (Switzerland) at 65°C, to obtain 150g of powder extract. The w/w yield of the prepared extract was 6%. The extract was stored at 4 °C until used.

Brine shrimp lethality assay

The eggs of Brine shrimp were procured from Philadelphia, USA. In a small water tank containing sea water, the eggs were incubated for 48 hours for hatching. Required light was provided with Philips 40 Watts lamp for 12 hours cycle. After 48 hours, the larvae were used for the experiments. The nauplii of Brine shrimp were challenged in different test tubes containing 10 mL of sea water and 20 larvae. To this, extracts of leaves at different concentrations (1, 2 3, 4 and 5 mg/mL) were added. After 48 hours, the viability of larvae was observed and mortality was recorded. Nauplii were considered dead when they were immobile and stayed at the bottom of the test tubes. The percent mortality of brine shrimp was calculated with the formula given below.

[% mortality = No.of brine shrimp dead /No.of brine shrimp introduced x 100]

Table 1: Brine shrimp lethality assay of *Acacia catechu* seed extract

Sample	Incubation Period	Concentration(mg/ml)				
		100 µl /ml	2 00 µl /ml	300 µl /ml	400 µl mg/ml	500 µl /ml
Acacia catechu *seed extract*	24 h	50	55	100	100	100
	48 h	50	75	100	100	100

RESULT AND DISCUSSION

The present study was conducted to evaluate in vitro brine shrimp lethality assay of ethonolic extract of Acacia catechu seed and correlate cytotoxicity results with known pharmacological actions of the plant. The results obtained from different concentrations of brine shrimp lethality assay are given in Table1. It shows that Acacia catechu extract is highly toxic to brine shrimp with increase in concentration. It was observed that at a concentration of 100 µl / ml 50% of mortality is seen in brine shrimp in 24 and 48 hours. At 200 µl /ml concentration 55% of brine shrimp were killed in 24 hours and 75% were killed in 48 hours, at 300 µl / ml concentration 100% of the brine shrimp were killed and at 24 & 48 hours. At a concentration of 400 µl /ml 100%mortality of brine shrimp was noticed in 24 hours and at 48

hours even, at 500 µl / ml concentration about 100% of brine shrimp were killed and these concentration proves the larvicidal efficacy of the Acacia catechu seed ethanolic extract.The results were tabulated in table 1 and depicted in fig 1.

CONCLUSION

Seed of Acacia catechu extract possesses lots of medicinal value. Brine shrimp lethality assay is an economical and cost effective method for investigating cytotoxic activity. Hence from the results it can be concluded that Acacia catechu seed is a promising candidate against brine shrimp and possesses cytotoxic activity.

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CONFLICT OF INTEREST

Nil

REFERENCES

1. Roy A, Lakshmi T, Geetha RV, et al. Estimation of Quercetin in Acacia catechu ethanolic bark extract by HPLC method. *International Journal of PharmTech Research* 2012; 4: 501–505.
2. Singh KN, Lal B. Notes on Traditional Uses of Khair (Acacia catechu Willd.) by Inhabitants of Shivalik Range in Western Himalaya. *Ethnobotanical Leaflets* 2006; 10: 109–112.
3. Naik GH, Priyadarsini KI, Satav JG, et al. Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine. *Phytochemistry* 2003; 63: 97–104.
4. Lakshmi T, Anitha R, Geetha RV. Acacia catechu willd-A gift from Ayurveda to mankind-A review. *T Ph Res* 2011; 5: 273–293.
5. Lakshmi T, S AK. Preliminary phytochemical analysis & Invitro Antibacterial activity of Acacia catechu willd Bark against Streptococcus mitis, Streptococcus sanguis & Lactobacillus acidophilus. *Phytomedicine* 2012; 3: 579–584.
6. Devaraj E, Lakshmi T. Cytotoxic and Apoptotic Effects of Acacia catechu in Hepatocellular Carcinoma HepG2 Cells. *Journal of Clinical and Experimental Hepatology* 2017; 7: S76.
7. Ezhilarasan D, Lakshmi T, Nagaich U, et al. Acacia catechu ethanolic seed extract triggers apoptosis of SCC-25 cells. *Pharmacognosy Magazine* 2017; 13: 405.
8. Ezhilarasan D, Lakshmi T, Vijayaragavan R, et al. Acacia catechu ethanolic bark extract induces apoptosis in human oral squamous carcinoma cells. *Journal of Advanced Pharmaceutical Technology & Research* 2017; 8: 143.
9. Alambayan J, Vats M, Sardana S, et al. Evaluation of antiulcer activity of roots of Acacia catechu Willd.(Mimosoideae). *Journal of Pharmacognosy and Phytochemistry* 2015; 3: 79–84.
10. Lakshmi T, Magesh A, Rajendran R. Estimation of Biomarker Epicatechin in Ethanolic Bark Extract of Acacia catechu willd by HPLC Method, http://www.pharmainfo.in/jpsr/Documents/Volumes/vol4Issue03/jpsr%2004120304.pdf.
11. Jain R, Patni V, Arora DK. Isolation and identification of flavonoid 'quercetin' from Acacia catechu (L.F.) Willd - a Katha yielding plant. *Journal of Phytological Research* 2007; 20: 43–45.
12. Lakshmi T, Rajendran R, Madhusudhanan N. Chromatographic fingerprint analysis of Acacia catechu ethanolic leaf extract by HPTLC Technique. *Int J Drug Development and Research* 2012; 4: 180–185.
13. Negi BS, Dave BP. In Vitro Antimicrobial Activity of Acacia catechu and Its Phytochemical Analysis. *Indian J Microbiol* 2010; 50: 369–374.
14. Ghate NB, Hazra B, Sarkar R, et al. Heartwood extract of Acacia catechu induces apoptosis in human breast carcinoma by altering bax/bcl-2 ratio. *Pharmacogn Mag* 2014; 10: 27–33.
15. Meyer BN, Ferrigni NR, Putnam JE, et al. Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Med* 1982; 45: 31–34.
16. Lakshmi T, Ramasamy R, Thirumalaikumaran R. Preliminary Phytochemical analysis and In vitro Antioxidant, FTIR Spectroscopy, Anti-diabetic activity of Acacia catechu ethanolic seed extract. *Pharmacognosy Journal* 2015; 7: 356–362.
17. Sajan, D., Lakshmi, K. U., Erdogdu, Y. and Joe, I. H., Molecular structure and vibrational spectra of 2,6-bis(benzylidene)cyclohexanone: A density functional theoretical study, Spectrochimica Acta Part a-Molecular and Biomolecular Spectroscopy, 2011, 78(1):113-121.
18. Lekha, L., Raja, K. K., Rajagopal, G. and Easwaramoorthy, D., Schiff base complexes of rare earth metal ions: Synthesis, characterization and catalytic activity for the oxidation of aniline and substituted anilines, Journal of Organometallic Chemistry, 2014, 753:72-80
19. Patil, S. B., Durairaj, D., Kumar, G. S., Karthikeyan, D. and Pradeep, D., Comparison

of Extended Nasolabial Flap Versus Buccal Fat Pad Graft in the Surgical Management of Oral Submucous Fibrosis: A Prospective Pilot Study, Journal of Maxillofacial & Oral Surgery, 2017, 16(3):312-321

20. Wahab, P. U. A., Nathan, P. S., Madhulaxmi, M., Muthusekhar, M. R., Loong, S. C. and Abhinav, R. P., Risk Factors for Post-operative Infection Following Single Piece Osteotomy, Journal of Maxillofacial & Oral Surgery, 2017, 16(3):328-332
21. Eapen, B. V., Baig, M. F. and Avinash, S., An Assessment of the Incidence of Prolonged Postoperative Bleeding After Dental Extraction Among Patients on Uninterrupted Low Dose Aspirin Therapy and to Evaluate the Need to Stop Such Medication Prior to Dental Extractions, Journal of Maxillofacial & Oral Surgery, 2017, 16(1):48-52
22. Menon, S., Devi, K. S. S., Santhiya, R., Rajeshkumar, S. and Kumar, S. V., Selenium nanoparticles: A potent chemotherapeutic agent and an elucidation of its mechanism, Colloids and Surfaces B-Biointerfaces, 2018, 170:280-292.
23. Wahab, P. U. A., Madhulaxmi, M., Senthilnathan, P., Muthusekhar, M. R., Vohra, Y. and Abhinav, R. P., Scalpel Versus Diathermy in Wound Healing After Mucosal Incisions: A Split-Mouth Study, Journal of Oral and Maxillofacial Surgery, 2018, 76(6):1160-1164
24. Krishnamurthy, A., Sherlin, H. J., Ramalingam, K., Natesan, A., Premkumar, P., Ramani, P. and Chandrasekar, T., Glandular Odontogenic Cyst: Report of Two Cases and Review of Literature, Head & Neck Pathology, 2009, 3(2):153-158
25. Prasad, SV; Kumar, M; Ramakrishnan, M; Ravikumar, D Report on oral health status and treatment needs of 5-15 years old children with sensory deficits in Chennai, India,2018,38(1):58-59
26. Uthrakumar, R; Vesta, C; Raj, CJ; Krishnan, S; Das, SJ Bulk crystal growth and characterization of non-linear optical bisthiourea zinc chloride single crystal by unidirectional growth method,2010,10(2):548-552.
27. Ashok, BS; Ajith, TA; Sivanesan, S Hypoxia-inducible factors as neuroprotective agent in Alzheimer's disease2017,44(3):327-334
28. Neelakantan, P; Sharma, S; Shemesh, H; Wesselink, PR Influence of Irrigation Sequence on the Adhesion of Root Canal Sealers to Dentin: A Fourier Transform Infrared Spectroscopy and Push-out Bond Strength Analysis,2015,41(7):1108-1111.
29. Haribabu, K; Muthukrishnan, S; Thanikodi, S; Arockiaraj, GA; Venkatrama, Investigation Of Air Conditioning Temperature Variation By Modifying The Structure Of Passenger Car Using Computational Fluid Dynamics,2020,24(1):495-498