

Comparative Study on the Brine Shrimp Lethality Assay of Hexane, Ethyl Acetate & Methanol Extracts of *Maytenus senegalensis* Stem Part & *Annona muricata* Aerial Part

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Abstract: Medicinal plants are known to contain different bioactive components with enormous therapeutic potential to cure many diseases in human beings. In this research study two different medicinal plants that are used for the treatment of various illnesses in South West Nigeria, were subjected to toxicity test using brine shrimp lethality test at different concentrations. The percentage mortality increases as the extract concentration increases and none of the extracts exhibited higher percentage mortality than the standard drugs used. The hexane extract of *Annona muricata* exhibited higher percentage mortality at higher concentrations than the hexane extract of *Maytenus senegalensis*. The ethyl acetate extracts of the two plants had the same percentage mortality at 60.00 µg/ml, 80.00 µg/ml and 100.00 µg/ml concentrations. The *Annona muricata* methanol extract showed better toxicity than *Maytenus senegalensis* methanol extract at all concentrations. It exhibits the most significant cytotoxicity activity against brine shrimp (*Artemia salina*) having the lowest lethal concentration (LC₅₀) value of 32.515 µg/ml. The two medicinal plants have demonstrated good cytotoxicity activity and it is an indication that they can exhibit good anticancer property when tested against some cancer cell lines.

Keywords: Medicinal plants, Percentage mortality, Toxicity, Anticancer property, Lethal, concentration.

1. INTRODUCTION

Background of the Study

Medicinal plants are valuable resources for our health care system since ancient period. These natural herbs are an important source of drugs for alternative medicine systems and they are thought to constitute 80 % of total drugs used by humans. These natural healing herbs are used in the treatment of many diseases and disorders (Natural Remedies Home, 2020). *Maytenus senegalensis* is a synonym of *Celastrus senegalensis* and it has been reported to have antibacterial property (Lindsey *et al.*, 2006, Jain *et al.*, 2008). An extract of the stem-bark of *M. senegalensis* demonstrated *in vitro* antileishmanial activity against promastigotes of *Leishmania major* reference vaccine strain (5AKSH) (El Tahir *et al.*, 1998). Pristimerin was identified as the antileishmanial agent of *M. senegalensis* and proved to be active against resistant strains of *P. falciparum*. (Matu *et al.*, 2003).

A. muricata is native to the warmest tropical areas in South and North America and is now widely distributed throughout tropical and subtropical parts of the world, including India, Malaysia and Nigeria (Adewole and Coxton-Martins, 2006). *A. muricata* has a strong ability to selectively inhibit cancer cells growth (Baskar *et al.*, 2007; Viera *et al.*, 2010). Currently, the increasing use of ethnobotanical *A. muricata* extract as an anticancer therapy due to the selective cytotoxic activity of the plant as a growth inhibitor for the tumor cells without affecting the healthy cells (George *et al.*, 2012). Brine shrimp lethality assay (BSLA) is a simple, high throughput cytotoxicity test of bioactive chemicals and natural

products (Meyer *et al.*, 1982). For the bioactive compound of either natural or synthetic origin, this is a rapid and comprehensive test. It is also an inexpensive and simple test as no aseptic techniques are required. (Quazi *et al.*, 2017). The main objective of this research work was to investigate the toxicity potential of these medicinal plants as claimed by the traditional people that they have ability to cure cancer related diseases.

2. MATERIALS &METHODS

2.1 Plant Collection

The fresh stem part of *Maytenus senegalensis* and aerial part of *Annona muricata* were obtained locally from farmlands in Ogun State and Lagos State, South West, Nigeria. The plant materials were air dried under shade, grinded to coarse powder and stored in a tight container until use.

2.2 Preparation of Extracts

The grinded plant material was sequentially extracted using hexane, ethyl acetate and methanol respectively using the method of maceration at normal room temperature for a period of three days according to Handa *et al.*, 2008. The extract was filtered and then distilled off the extracting solvent by drying it on an evaporating dish under a mild temperature.

2.3 Brine Shrimp Lethality Test

The lethal concentration (LC₅₀) was determined using Brine Shrimp Lethality Test (BSLT) as to determine the level of bio-activity of the extracts at different concentrations of application. Brine Shrimp (*Artemia salina*) were hatched using brine Shrimp eggs in a vessel filled with sterile artificial seawater under constant aeration for 48 hrs. After hatching active nauplii free from egg shell were collected from brighter portion of the hatching chamber and used for the assay. Ten nauplii were drawn through a glass capillary and placed in each vial containing 4.5 ml of brine solution to give different concentration (20.00, 40.00, 60.00, 80.00 and 100.00 µg/ml) and maintained at room temperature for 24 hrs under the light and surviving larvae were counted. Experiments were conducted along with control and test substances in a set of three tubes per dose.

3. RESULTS & DISCUSSION

Table 1: Percentage Mortality Rate of Brine Shrimps at Different Concentrations of *Annona muricata* Aerial Part Extracts

Extract	Concentration (µg/ml)	No Exposed	No Responded	% Mortality	LC ₅₀ (µg/ml)
Hexane Extract	20.00	20.00	8.00	40.00	38.577
	40.00	20.00	10.00	50.00	
	60.00	20.00	14.00	70.00	
	80.00	20.00	15.00	75.00	
	100.00	20.00	16.00	80.00	
Ethyl Acetate Extract	20.00	20.00	7.00	35.00	52.875
	40.00	20.00	9.00	45.00	
	60.00	20.00	11.00	55.00	
	80.00	20.00	13.00	65.00	
	100.00	20.00	15.00	75.00	
	20.00	20.00	10.00	50.00	
	40.00	20.00	11.00	55.00	

Methanol Extract	60.00	20.00	12.00	60.00	32.515
	80.00	20.00	15.00	75.00	
	100.00	20.00	15.00	75.00	
Standard (K₂Cr₂O₇)	25.00	20.00	17.00	85.00	5.653
	100.00	20.00	20.00	100.00	
Standard (Thymol)	12.50	20.00	20.00	100.00	0.531
	25.00	20.00	20.00	100.00	
	100.00	20.00	20.00	100.00	

Table 2: Percentage Mortality Rate of Brine Shrimps at Different Concentrations of *Maytenus senegalensis* Stem Part Extracts

Extract	Concentration (µg/ml)	No Exposed	No Responded	% Mortality	LC₅₀ (µg/ml)
Hexane Extract	20.00	20.00	10.00	50.00	41.045
	40.00	20.00	11.00	55.00	
	60.00	20.00	11.00	55.00	
	80.00	20.00	12.00	60.00	
	100.00	20.00	13.00	65.00	
Ethyl Acetate Extract	20.00	20.00	7.00	35.00	50.430
	40.00	20.00	10.00	50.00	
	60.00	20.00	11.00	55.00	
	80.00	20.00	13.00	65.00	
	100.00	20.00	15.00	75.00	
Methanol Extract	20.00	20.00	7.00	35.00	53.247
	40.00	20.00	9.00	45.00	
	60.00	20.00	10.00	50.00	
	80.00	20.00	13.00	65.00	
	100.00	20.00	16.00	80.00	
Standard (K₂Cr₂O₇)	25.00	20.00	17.00	85.00	5.653
	100.00	20.00	20.00	100.00	
Standard (Thymol)	12.50	20.00	20.00	100.00	0.531
	25.00	20.00	20.00	100.00	
	100.00	20.00	20.00	100.00	

Brine Shrimp Lethality Test

The cytotoxicity properties of the extracts were determined by subjecting them to brine shrimp lethality test at different extract concentrations. The cytotoxicity properties of the two medicinal plants were compared to the known standard drugs (Thymol and Potassium dichromate) at different concentrations and their respective lethal concentration (LC₅₀) were determined. The results showed that the percentage mortality rate increases with increase in concentration and none of the plant extracts exhibited better cytotoxicity property than the standard drugs. In Table 1, the hexane, ethyl acetate and methanol extracts of *Annona muricata* at 100.00 (µg/ml) concentration gave the same percentage mortality rate (75.00 %). The LC₅₀ of the *Annona muricata* extracts ranged from 32.515 µg/ml to 52.876 µg/ml, while the methanol extract had the least LC₅₀ value.

In Table 2, the percentage mortality rate of brine shrimps at different concentrations of *Maytenus senegalensis* stem part extracts are shown. The methanol extract of *Maytenus senegalensis* at 100.00 µg/ml concentration gave the highest percentage mortality rate, it had 80.00 % mortality rate. The LC₅₀ of *Maytenus senegalensis* extracts ranged from 41.045 µg/ml to 53.247 µg/ml, while the hexane had the least LC₅₀ value. In all the concentrations, the standard drug (Thymol) led to the death of all the brine shrimps, having 100.00 % mortality rate.

4. DISCUSSION

Medicinal plants are known to be source of bioactive compounds that have therapeutic values, the World Health Organization (WHO) encourages the inclusion of herbal medicine in health care because of the great potential they possess (Amos *et al.*, 2001). In this study, the percentage mortality rate of brine shrimps at different concentrations of *Annona muricata* and *Maytenus senegalensis* extracts were compared with the standard drugs. In Table 1, the ethyl acetate and methanol extracts of *Annona muricata* at 100.00 µg/ml concentration gave the same percentage mortality rate (75.00 %). In Table 2, the hexane extract of *Maytenus senegalensis* stem part showed higher cytotoxicity activity than the other *Maytenus senegalensis* extracts at the lower concentrations. Acute and sub-acute toxicity screening of *M. heterophylla* and *M. senegalensis* ethanol extracts (70 %) was evaluated in adult male CD-6 mice. While *M. heterophylla* extract at 1200 mg/kg is shown to be non-toxic, *M. senegalensis* extract indicated some toxicity (da Silva *et al.*, 2011).

The chloroform, hexane and methanolic extracts of *M. senegalensis* roots present ant-inflammatory activity, decreasing ear edema induced by croton oil in mice (Sosa *et al.*, 2007). *M. heterophylla* and *M. senegalensis* have been used in African traditional medicine for the treatment of numerous ailments, including respiratory diseases, inflammation, microbial affections and topical application for healing wounds (da Silva *et al.*, 2011). Using *in vivo* studies, extracts and phytochemicals of *A. muricata* have been characterized as an antimicrobial, anti-inflammatory, anti-protozoan, antioxidant, insecticide, larvicide and cytotoxic to tumor cells (Coria-Tellez *et al.*, 2018). Ethanol and water extracts of *Annona muricata* L. leaves and pearl grass was subjected to evaluate the cytotoxic activity against the human breast cancer. Ethanolic extract of *Annona muricata* L. leaves with IC₅₀ values 14.678 µg/ml and 88.788 µg/ml respectively displayed strongest cytotoxic activity against the Michigan Cancer Foundation-7 (MCF-7) on 24 and 48 hours of experiment as compared to water extract show least cytotoxic activity (Endrini *et al.*, 2015; Najmuddin *et al.*, 2016).

5. CONCLUSION

In this research study, *Annona muricata* extracts and *Maytenus senegalensis* extracts were active against the brine shrimp, having good LC₅₀ values due to the presence of the cytotoxicity compounds in them. Therefore, it an indication that those bioactive compounds present in the plant extracts may exhibit anticancer property when tested against some cancer lines. Therefore, further research is essential as to determine the anticancer property of the bioactive compounds present in the extracts using different cancer cell lines.

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