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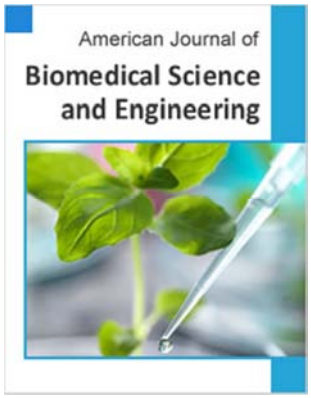

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Correlation Between Active Components of Rocket (*Eruca sativa*) as Cytotoxicity (Brine Shrimp Lethality Assay)

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Abstract
Eruca sativa Miller of the family Brassicaceae is grown in West Asia and North Africa as poor quality oilseed crop at peripheral land under poor rainwater. Numerous human pathogenic bacteria and fungi have been subdued into drug-resistant strains. Countless synthetic antioxidant compounds have posted carcinogenic effects. This phenomenon requests further research for new effective drugs of natural origin. This manuscript aimed to screen new source of biologically active compounds from rocket plant. In Brine Shrimp Lethality Bioassay, all the extracts produced dose-dependent cytotoxicity effect to brine shrimp nauplii with methanol extract of seed exhibiting highest toxicity. Cytotoxicity was evaluated in terms of LC50 (lethality concentration). The final concentrations were 1000, 100, 10 and 1 ppm (µg/mL). Ten nauplii were added into (3) replicates of each concentration of the plant extract. After 24 hours the surviving brine shrimp larvae were counted and LC50 was assessed. Results showed that the extracts of *E. sativa* meal (methanolic, Hexane, Heat treatment-methanol), and oil were potent against the brine shrimp with LC50 values of 91, 106, 111 and 98 ppm (µg/mL), respectively. It indicated that bioactive components are present in these plants that could be accounted for its pharmacological effects. Thus, the results support the uses of these plant species in traditional medicine. *E. Sativa* meal and oil have the highest of total polyphenols and flavonoids contents, which were 49.77 mg GAE/g and 11.82 mg GAE/g, respectively. Antioxidant activity of methanolic, hexane and heat-methanolic extracts and oil of *E. saliva* were determined by using FRAP, ABTS, and DPPH.

1. Introduction

Some of the traditional medicine includes the use of crude plant extracts which may contain an extensive diversity of molecules, often with indefinite biological effects [1]. However, most of the available information regarding the medicinal potential of these plants is not delivered with credible scientific data. For this reason, several types of

research have been steered to determine the toxicity of medicinal plants [2]. A general bioassay that appears capable of detecting a broad spectrum of bioactivity present in plant crude extracts is the Brine Shrimp (*Artemia*sp.) Lethality Assay (BSLA) [3]. BSLA is used as an indicator for general toxicity and also as a guide for the detection of antitumor and pesticidal compounds [4]. The low cost and ease of performing the assay and the commercial availability of inexpensive brine shrimp eggs make BSLA a very useful bench top method [5]. This assay has been noted as a useful tool for the isolation of bioactive compounds from plant extracts [6].

Olowa and Nuñeza [7], the study was accompanied to test for in vivo Brine Shrimp Lethality Assay (BSLA) of the ethanolic extracts of Lantanacamara, *Chromolaenaodorata*, and *Euphorbia hirta* and *correlate cytotoxicity* results with known pharmacological activities of the plants. Novel cytotoxic, antitumor, and pesticidal compounds can be isolated from potential plant sources through the assessment of cytotoxic activity against brine shrimps. Cytotoxicity was evaluated in terms of LC_{50}(lethality concentration). Ten nauplii were added into three replicates of each concentration of the plant extract. After 24 hours the surviving brine shrimp larvae were counted and LC_{50} was assessed. Results showed that the extracts of *L. camara*, *C.odorata*, and *E. hirta* were potent against the brine shrimp with LC_{50} values of 55, 10, and 100 ppm (µg/mL), respectively. It indicated that bioactive components are present in these plants that could be accounted for its pharmacological effects. Thus, the results support the uses of these plant species in traditional medicine.

2. Material and Methods

2.1. Preparation of Plant Extracts

The final concentrations were 1000, 100, 10, and 1ppm (µg/mL). There were three (3) replicates in each concentration. A control test was also prepared.

2.2. Brine Shrimp Lethality Assay (BSLA)

Brine shrimp eggs were obtained from the New Aqua Laboratory in Naawan, Misamis Oriental, as a gift sample for the research work. Filtered, artificial seawater was prepared by dissolving 38 g of sea salt in 1 liter of distilled water for hatching the shrimp eggs. The seawater was put in a small plastic container (hatching chamber) with a partition for dark (covered) and light areas. Shrimp eggs were added to the dark side of the chamber while the lamp above the other side (light) will attract the hatched shrimp. Two days were allowed for the shrimp to hatch and mature as nauplii (larva). After two days, when the shrimp larvae are ready, 4 mL of the artificial seawater was added to each test tube and 10 brine shrimps were introduced into each tube. Thus, there was a total of 30 shrimps per dilution. Then the volume was adjusted with artificial seawater up to 5 mL per test tube. The test tubes were left uncovered under the lamp. The number of surviving shrimps were counted and recorded after 24 hours. Using probit analysis, the lethality concentration (LC_{50}) was assessed at 95% confidence intervals. LC_{50} of less than 100 ppm was considered as potent (active) [8]. As mentioned by Meyer *et al.* [4], theLC_{50} value of less than 1000 µg/mL is toxic while the LC_{50} value of greater than 1000 µg/mL is non-toxic. The percentage mortality (%M) was also calculated by dividing the number of dead nauplii by the total number and then multiplied by 100%. This is to ensure that the death (mortality) of the nauplii is attributed to the bioactive compounds present in the plant extracts.

2.3. Determination of Antioxidant Factors

Total phenolic contents of the air dried meal and oil were determined by using Folin-Ciocalteu reagent method according to [9]. Flavonoids are polyphenolic compounds comprising fifteen carbons with two aromatic rings connected by a three-carbon bridge, hence C6-C3-C6. They are the most numerous of the phenolics and are found throughout the plant kingdom [10, 11]. Total flavonoids content of the air dried meal and oil were determined colorimetrically using aluminum chloride as described by Chang *et al.* [12]. Reducing the power of methanolic flowers extracts was determined according to the method of Oyaizu [13]. ABTS (2,2'-azino-*bis*(3-ethyl benzothiazoline-6-sulfonic acid) assay was based on the method of Re *et al.* [14]. The DPPH free radical scavenging activity of Asteraceae *(Family Compositae)* flowers extracts at different concentrations were measured from bleaching of the purple color of the (2.2-diphenyl-1-picryl hydroxyl) *assay* was based on the method of Pratap *et al.* [15].

3. Results and Discussions

The three extracts and oil of plant tested showed good brine shrimp larvicidal activity. The lethality concentration (LC_{50}) of methanolic, Hexane, Heat treatment-methanol extracts and oil *E. sativa* were 91 ppm (µg/mL), 106 ppm, 111 ppm, and 98 ppm respectively (Table 1). The extracts and oil of *Eruca sativa* (taramira) tested showed good brine shrimp larvicidal activity (Figure. 1). The degree of lethality was directly proportional to the concentration of the extracts and oil. Maximum mortalities (100%) were observed at a concentration of 1000 ppm in both *methanol*, *hexane, Heat treatment-methanol* extracts and oil of *Eruca sativa*. Based on the results, the brine shrimp lethality of the three plant extracts was found to be concentration-dependent. The observed lethality of the three extracts and oil to brine shrimps indicated the presence of potent cytotoxic and probably antitumor components of these plant. According to Meyer *et al.* [4], crude plant extract is toxic (active) if it has an LC_{50} value of fewer than 1000 µg/mL while non-toxic (inactive) if it is greater than 1000 µg/mL. All the extracts were also subjected to Brine Shrimp lethality bioassay for possible cytotoxic action. In this, the study, the methanol

extract of the aerial part was found to be the most toxic to Brine Shrimp nauplii.

Saha*et al.*, [16], have investigated that the cytotoxic and anthelmintic activities of *Leonurussibiricus* L. (commonly known as Raktodrone in Bangladesh) belonging to the family Labiatae. The dried leaves and roots of *L. sibiricus* were extracted with methanol and fractionated by modified Kupchan method. The crude methanolic extracts as well as its soluble fractions of petroleum ether, ethyl acetate and chloroform were screened for cytotoxic activity using brine shrimp lethality bioassay. They were found to possess significant cytotoxic activities. The LC_{50} values of crude extract of leaves and its pet-ether, ethyl acetate and chloroform-soluble fractions were 1.0, 2.0, 2.11 and1.33 µg/mL, respectively. On the other hand, the LC_{50} of crude methanolic extract of roots and fractions of pet-ether, ethyl acetate and chloroform were 2.0 µg/mL, 2.81 µg/mL, 3.55 µg/mL and 7.58 µg/mL, respectively. Vincristine sulfate was used as positive control. The crude methanol extract of leaves and roots also showed very good anthelmintic activities as determined against the earthworms, *Pheretimaposthuma*. The study confirms the moderate anthelmintic and potent cytotoxic activities of leaf and root extracts of *L. sibiricus*.

Table (2) showed the total polyphenols (mgGAE/g) and total flavonoids (mgQE/g) contents of *Eruca sativa* meal extract and oil. It was clear that, the Methanol extract of the meal and oil *Eruca sativa* have the highest concentration of total polyphenols, was 49.77 and 44.22 mg GAE/g, respectively. While meal with heat treatment has the low concentration of total polyphenols, was 26.11mg GAE/g. Also, Hexane extracts of *E. Sativa meal* containing medium values of total polyphenols which were 31.44 mg GAE/g. Total flavonoids as shown in Table (2) ranged from 4.59 to 11.82 mgQE/g dry weight for Heat treatment and Methanol extract of *E. sativa* meal, respectively. *E. saliva* has the highest reducing power which was 2.0191, 1.4765formethanolic extract and oil at the concentrations of 80 mg/ml, respectively. Moreover, the capacity of heat treatment, hexane extracts, oil, and methanol extract to scavenge the ABTS radical was 41.49, 59.67, 65.23 and 72.19%, respectively [17]. The antioxidant activity of the tested extracts was measured using DPPH radical scavenging activity. The antioxidants scavenging activities for DPPH are attributed to their hydrogen-donating abilities [18]. Vitamin C was used as the reference compound.

Table 1. *The number of shrimp nauplii that survived after treating with the four extracts of roquette meal and LC_{50} obtained by Brine Shrimp.*

Eruca sativa	Concentration (ppm or µg/mL)	Number of Surviving Nauplii after 24 h			Total Number ofSurvivors	LC_{50} (mg/mL) %Mortality
		T1*	T2	T3		
Methanol extract	1	8	8	9	25	0
	10	4	4	6	14	39
	100	3	3	4	10	55
	1000	0	0	0	0	100
Hexane extract	1	8	9	8	25	0
	10	5	6	5	16	31
	100	4	4	4	12	47
	1000	0	0	0	0	100
Heat treatment-methanol extract	1	8	9	9	26	0
	10	6	5	7	18	29
	100	5	4	5	14	45
	1000	0	0	0	0	100
Oil	1	9	9	8	26	0
	10	6	5	4	15	35
	100	4	4	3	11	51
	1000	0	0	0	0	100

* T= Trial.

Table 2. *Total polyphenols (mg GAE/g), total flavonoids (mg QE/g), Reducing power as absorbency at 700 nm concentrations 80 mg/ml, and Antioxidant capacity of Transactions determined by(ABTS+) and (DPPH) action radical contents of Transactions.*

Transactions	Total polyphenols (mgGAE/g)	Total flavonoids (mgQE/g)	Reducing power (FRAP)	($ABTS^+$) action radical, % Inhibition	IC_{50} (DPPH), 250 µg/mL
Methanol extract	49.77	11.82	2.0191	72.19	0.059
Hexane extract	31.44	7.77	1.1719	59.67	0.031
Heat treatment	26.11	4.59	0.9409	41.49	0.012
Oil	44.22	9.70	1.4765	65.23	0.047

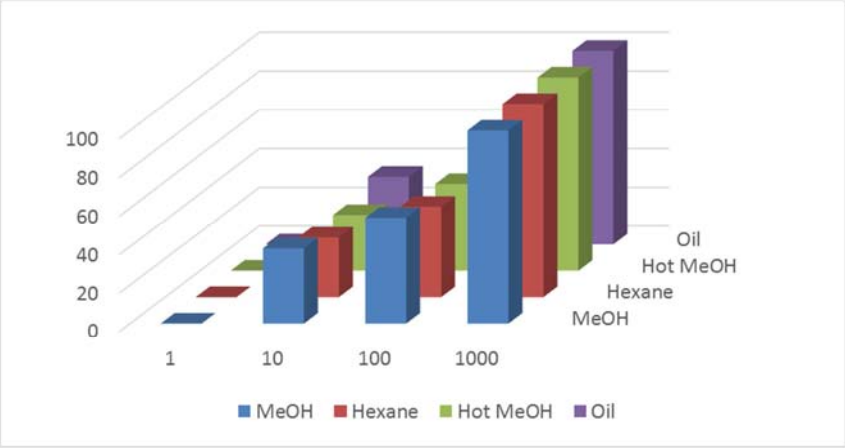

Figure 1. *Comparison of LC_{50} (mg/mL) values of each extract against different concentrations.*

Syahmi *et al.* [19], have mentioned that *Elaeisguineensis* (Arecaceae) is widely used in West African traditional medicine for treating various ailments. An evaluation of the toxicity of extracts of this plant is crucial to support the therapeutic claims. The acute oral toxicity and brine shrimp lethality of a methanolic extract of this plant were tested. Oral administration of crude extract at the highest dose of 5,000 mg/Kg resulted in no mortalities or evidence of adverse effects, implying that *E. guineensis*is nontoxic. Normal behavioral pattern, clinical signs, and histology of vital organs confirm this evidence. The *E. guineensis*extracts screened for toxicity against brine shrimp had 50% lethal concentration (LC_{50}) values of more than1.0 mg/mL (9.00 and 3.87 mg/mL at 6 and 24 h, respectively), confirming that the extract was not toxic. Maximum mortalities occurred at 100 mg/mL concentration while the least mortalities happened to be at 0.195 mg/mL concentration. The results of both tests confirm that *E. guineensis*is nontoxic and hence safe for commercial utilization.

Olowa and Nuñeza [7], have conducted to test for in vivo Brine Shrimp Lethality Assay (BSLA) of the ethanolic extracts of *Lantanacamara*, *Chromolaenaodorata*, and *Euphorbia hirta* and correlate cytotoxicity results with known pharmacologicalactivities of the plants. Novel cytotoxic, antitumor, and pesticidal compounds can be isolated from potential plant sources through the assessment of cytotoxic activity against brine shrimps. Cytotoxicity was evaluated in terms of LC_{50} (lethality concentration). Ten nauplii were added into three replicates of each concentration of the plant extract. After 24 hours the surviving brine shrimp larvae were counted and LC_{50} was assessed. Results showed that the extracts of *L. camara*, *C. odorata*, and *E. hirta* were potent against the brine shrimp with LC_{50} values of 55, 10, and 100 ppm (µg/mL), respectively. It indicated that bioactive components are present in these plants that could be accounted for its pharmacological effects. Thus, the results support the uses of these plant species in traditional medicine.

4. Conclusion

The experimental results presented that rocket seed extracts and oil could be potential sources of various biologically active natural compounds. Different crude extracts of *Erucasativa* were subjected to cytotoxic vigorous phytochemical and pharmacological investigations to validate the traditional use and to find out any other therapeutic activities. The high toxicity exerted by the extracts of taramira seeds and oil in brine shrimp lethality bioassay suggests bioactive principles in the plant. The leaf of the plant exhibited potential antioxidant activity. This part of the plant exerted remarkable analgesic and anti-inflammatory activity. This is because their LC_{50} values are less than 1000 ppm or µg/mL. The ethnopharmacological activities of these plant species are due to the different bioactive compounds present in these plants. Although BSLA is inadequate in regulating the mechanism of action of the bioactive substances in the plant, it is very useful by delivering a preliminary screen that can be supported by a more specific bioassay, once the active compound has been isolated. Thus, some useful drugs of therapeutic importance may develop out of the research work.

References

[1] Konan N. A.; Bacchi E. M.; Lincopan N.; Varela S. D.;Varanda E. A. (2007). Acute, subacute toxicity and genotoxic effect of a hydroethanolic extract of the cashew (*Anacardiumoccidentale* L.), *J. Ethnopharmacol*, 110:30-38.

[2] Sasidharan S.; Badakhshan M. P.; Rameshwar N. J.; Ramanathan S. (2009). Comparative study: antimicrobial activity of methanol extracts of *Lantana camera* various parts, *Pharmacogn Res*, 1:348-351.

[3] Pisutthanan S.; Plianbangchang P.; Pisutthanan N.; Ruanruay S; Muanrit O. (2004). Brine shrimp lethality activity of Thai medicinal plants in the family Meliaceae, *Naresuan University Journal*, 12(2):13-18.

[4] Meyer B. N.; Ferrigni N. R.; Putnam J. E.; Jacobsen L. B.; Nichols D. E.; McLaughlin J. L. (1982). Brine shrimp: A convenient general bioassay for active plant constituents, *Plant Med*, 45:31-34.

[5] McLaughlin J. L.; Chang C. J.; Smith D. L. (1991). Benchtop bioassays the discovery of bioactive natural products: an update, *Nat Prod Chem*, 9:383-397.

[6] Sam T. W. (1993). Toxicity testing using the brine shrimp *Artemia salina* in: Bioactive natural products detection, isolation, and structural determination, CRC Press, Boca Raton: FL., pp. 442-456.

[7] Olowa, L. F.; Nuñeza, O. M. (2013). Brine Shrimp Lethality Assay of the Ethanolic Extracts of Three Selected Species of Medicinal Plants from Iligan City, Philippines. *Int. Res. J. Biol. Sci.,* 2(11):74-77.

[8] Gupta M. P.; Monge A.; Karitas G.; Lopez de Cerain A.; Solis P. N.; Leon E.; de Trujillo M.; Surez O.; Wilson F.; Montenegro G.; Noriega Y.; Santana A. I.; Correa M.; Sanchez C. (1996). Screening of Panamanian medicinal plants for brine shrimp toxicity, crown gall tumor inhibition, cytotoxicity and DNA interaction, *Int. J. Pharmacol*, 34:123-127.

[9] Lin, J-Y.; Tang, C-Y. (2007). Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. Food Chem., 101:140-147.

[10] Crozier, A.; Jaganath, I. B.; Clifford, M. N. (2006). Phenols, polyphenols, and tannins: An overview. In Plant Secondary Metabolites: Occurrence, Structure, and Role in the Human Diet; Crozier, A., Clifford, M., Ashihara, H., Eds.; Blackwell: Oxford, UK, pp. 1-24.

[11] Pereira, D. M.; Valentao, P.; Pereira, J. A.; Andrade, P. B. (2009). Phenolics: From Chemistry to Biology. Molecules, 14:2202-2211.

[12] Chang, C. C.; Yang, M. H.; Wen, H. M.; Chern, J. C. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J. Food Drug Anal., 10:178-182.

[13] Oyaizu, M. (1986). Studies on products of browning reaction: anti-oxidative activities of products of browning reaction prepared from glucosamine, Jpn. J. Nutr., 44:307-315.

[14] Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans C. (1999). Antioxidant activity applying an improved ABTS radical. cation decolorization assay. Free Radical Biol. Med. 26:1231-1237.

[15] Pratap, C. R.; Vysakhi, M. V.; Manju, S.; Kannan, M.; Abdul, K. S.; Sreekumaran, N. A. (2013). In vitro free radical scavenging activity of Aqueous and Methanolic leaf extracts of Aegletamilnadensis (Rutaceae). Int. J. Pharm Sci., 819-823.

[16] Saha, M. R.; Bari, F. M. S. N. Ul.; Rahman, M. A.; Islam, M. A. (2012). *In Vitro* Cytotoxic and Anthelmintic Activities of Leaf and Root Extracts of *Leonurussibiricus L. J. Sci. Res*. 4(3):721-727.

[17] El-Fadaly, H. A.; El-Kadi, S. M.; El-Moghazy, M. M.; Soliman, A. A. M.; El-Haysha, M. S. (2017). Antioxidant activity studies on extracts of Eruca sativa seed meal and oil, detoxification, the role of antioxidants in the resistant microbes. IJSRM Human J. 6(3):31-51.

[18] Biswas, M; Haldar, P. K.; Ghosh A. K. (2010). Antioxidant and free-radical-scavenging effects of fruits of Dregeavolubilis. J. Nat Sci. Biol. Med. 1(1):29-34.

[19] Syahmi, A. R. M.; Vijayaratna, S.; Sasidharan, S.; Latha, L. Y.; Kwan, Y. P.; Lau, Y. L.; Shin, L. N.; Chen, Y. (2010). Acute Oral Toxicity and Brine Shrimp Lethality of *Elaeisguineensis*Jacq., (Oil Palm Leaf) Methanol Extract. *Molecules*, 15:8111-8121; doi:10.3390/molecules15118111.