



## *In-vitro* Cytotoxicity, Anti-bacterial and Anti-inflammatory Activities of *Ecklonia radiata* and *Jania verrucosa* from Eastern Cape, South Africa

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### ABSTRACT

Seaweeds are marine floras constituting in part to over 90% of the oceanic biomass. Despite the enrichments of seaweeds, their potentials are largely unexplored. Hence, this study evaluated the phytochemical screening, antimicrobial, anti-inflammatory and cytotoxicity activities of methanolic extracts of *Ecklonia radiata* and *Jania verrucosa*. *E. radiata* and *J. verrucosa* were collected from sites on the rocky shores of the Mbashe and Port St. John rivers on the Eastern Cape coast of South Africa during spring low tides. Qualitative phytochemical screening was carried out using standard protocol. The assessment of anti-inflammatory activity was done by inhibition of albumin denaturation assay and antimicrobial analysis was performed against six different pathogenic clinical isolates. The cytotoxicity effect of the methanolic extracts were further investigated on MDA-MB-231, HepG2, CaCo-2 and KMST-6 by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay (MTT assay). Phytochemical analysis revealed the presence of tannins, phenols, glycosides, and alkaloid for both extracts. The methanolic extracts of both seed weeds also possess anti-inflammatory activity by inhibiting albumin denaturation. The average minimum inhibitory concentration (MIC) values of the plant extracts ranged from 160-10000 µg/mL. *J. verrucosa* methanolic extract had the most significant biological activity with the lowest MIC value (160 µg/ml). The cytotoxicity result showed that cells treated with the methanolic extracts of *E. radiata* and *J. verrucosa* have more cytotoxicity effect on MDA-MB-231 treated cells followed by HepG2 and CaCo-2. Non-cancerous cell line (KMST-6) however showed some selectivity in their activities. Among the cancer cell lines, HepG2 treated cells were significantly suppressed ( $p < 0.05$ ) with both extracts compared to 5µM camptothecin (standard drug). In conclusion, *E. radiata* and *J. verrucosa* have potential for chemoprevention of human breast, good anti-inflammatory and antibacterial activities.

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**Keywords:** Seaweeds, Anti-microbial, Anti-inflammatory, Phytochemical, Anti-cancer activity.

### Introduction

Natural products provide limitless prospects for novel drug leads because of the unmatched availability of chemical diversity.<sup>1</sup> In medicine, the use of local knowledge to identify new drugs against diseases or infections is a well-known approach.<sup>2</sup> Algae isolated from natural products have been widely explored by several tribes and ethnic groups since the beginning of time, as sources of food and medicine.<sup>3</sup> Secondary metabolites of marine organisms have also been used as insecticides, fragrance and pigments.<sup>4</sup> Oceans cover over 70% of the earth's surface, serve as habitat for up to 90% of the organisms on the planet and provides unique environments required by these organisms.<sup>5</sup> Over 9,000 documented new metabolites have been isolated from different marine organisms.<sup>6</sup>

Marine organisms serve as sources of several bioactive secondary metabolites with economic importance leading to successful drug development, production of animal feed, food supplements as well as raw materials for cosmetic and food industries.<sup>7</sup> The search for natural compounds from the oceans is compelled by their exceptional richness in unique secondary metabolites, which make them survive in a competitive environment.<sup>8</sup> These secondary metabolites could be used as chemical prototypes for drug discovery and development. Globally, seaweeds have attracted a lot of attention in Biotechnology and Pharmacology fields.<sup>9</sup> These properties however gives brown seaweeds a great value as potential sources of fucoidans and for the development of health promoting natural products.<sup>10</sup> Seaweeds serve as ingredients in foods especially in sushi, energy drinks, dairy products, soups, macaroni and cheese,<sup>11</sup> in many countries including China, Korea and Japan.<sup>12</sup> Fucan and alginic acid- the functional polysaccharides isolated from brown seaweed have shown to exhibit some biological potentials such as antiviral and anticoagulant properties and also some economical values especially in pharmaceutical and food industries.<sup>13</sup> Traditional Chinese, Egyptian and the Romans also relied on the properties of brown seaweed to treat a variety of ailments including wounds and burns,<sup>14</sup> while in the 18th century European physicians used it to treat stomach ulcers and cancer.<sup>15</sup> *E. radiata*, also referred to as kelp is a brown seaweed that is abundant in the sub-tidal regions of Northern and Eastern Coast of South Africa. Kelp is the largest and fastest-growing marine algae and grows up to 12 m tall. South Africa and Namibia are the only countries in the world that have

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natural kelp forests on its coast. Its stipe is hollow and full of gas to help it float. Kelp also has thick, strap like fronds that grow from a bulb near the surface while *J. verrucosa* is a slender-beaded coral weed belonging to a genus of red seaweeds with hard, calcareous, branching skeletons in the family *Corallinaceae*.<sup>16</sup> Medical science now uses corallines in the preparation of dental bone implants. It is also used as an additive in feed for cattle and pigs, as well as in the filtration of acidic drinking water.<sup>17</sup> Corallines are epilithic in nature and found on the lower shore of an aquatic ecosystem, below the littoral zone or on rocks near low tide level. However, they are occasionally epiphytic and found in rock pools, sublittoral fringe and sublittoral zone to at least 8 m depth. In South Africa, they are widely distributed in both Northern KwaZulu-Natal as well as Eastern Cape coast. It is a rich source of minerals, natural antioxidants, and bioactive metabolites with cosmeceutical potential. It is also a promising candidate for anti-ageing, skin whitening, skin conditioning and skin polishing cosmetics.<sup>5</sup> Information on phytochemical profile and biological activities of *E. radiata* and *J. verrucosa* found in Eastern coast of South Africa has not been fully exploited. In view of this, the study aimed to qualitatively assess the phytochemical constituents, evaluate the anti-inflammatory, antimicrobial, and cytotoxicity activities of the methanolic extracts of *E. radiata* and *J. verrucosa* on some clinical bacterial pathogens and selected cell lines respectively.

## Materials and Methods

### Materials

#### Micro-organisms

Microorganisms were kindly provided by Prof Mervin Meyer, Department of Biotechnology University of the Western Cape, Cape Town, South Africa. Methicillin-resistant *Staphylococcus aureus* (ATCC 29213), *Staphylococcus aureus* (ATCC 26923), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 25955), and *Streptococcus pyogenes* (ATCC 19615) were used in the study. These isolates were sub cultured on nutrient agar and the culture plate were incubated for 24 h at 37 °C. Sterile inoculating loop was used to pick five colonies of well isolated colonies of the different bacteria on the cultured plates and emulsified in 3 mL of physiological normal saline to a turbidity equivalent to 0.5 McFarland standard ( $1.5 \times 10^8$  CFU/mL).

#### Cell Lines

A panel of three cancer cell lines: Human liver carcinoma (HepG-2), Human breast adenocarcinoma (MDA-MD-231), Human epithelial colorectal adenocarcinoma cells (CaCo-2), and a non-cancerous cell line; Human fibroblast (KMST-6) were kindly provided by Prof. Mervin Meyer at the Department of Biotechnology, University of the Western Cape, Cape Town, South Africa. The cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% foetal bovine serum, and 1% penicillin–streptomycin in a 37 °C humidified incubator with 5% CO<sub>2</sub> saturation. Invitrogen Ltd., Grand Island, New York, supplied all cell culture reagents. Cells were seeded in 96-well cell culture plates at a cell density of  $2 \times 10^4$  cells per well.

#### Plant Samples Collection and Identification

The seaweeds were collected from two different rocky shores areas in East coast (Mbashe and Port St John rivers), Mthatha in South Africa during low tides. They were identified based on standard keys<sup>18</sup> and confirmed by Dr. Immelman, a taxonomist at the Department of Botany, Walter Sisulu University, Mthatha Campus, South Africa where the reference specimens were deposited with voucher numbers S02 and S07 for *E. radiata* and *J. verrucosa* respectively as seen in (Figure 1).

#### Sample Preparation

The seaweeds were washed with clean water to remove epiphytes and other marine organisms and rinsed with distilled water to remove the associated biota and salt debris, and then shade-dried at room temperature (25°C) for 3 weeks. The dried seaweed was powdered using a kitchen electric blender. The rotary evaporator used was a Heidolph model VV2000 equipped with a bump trap and a 500 mL round-bottom flask.

#### Preparation of Seaweed Extracts

Approximately 50 g of powdered seaweed was extracted by maceration in 500 mL of methanolic for 48 h with mild shaking at room temperature and filtered through a filter paper (Whatman, No. 42). The process was repeated three times to ensure complete extraction. The extracts were pooled together and concentrated in a rotary evaporator (Heidolph model vv2000 equipped with a bump trap and a 500 mL round-bottom flask). The concentrated extracts obtained were kept in a closed container and stored in the refrigerator before analysis.

#### Phytochemical screening

Phytochemical screening was carried out to detect the presence of tannins, flavonoids, phenols, saponins, glycosides, alkaloids, terpenoids, and phytosterols following the method described by Harborne (1984). Color change and/or precipitate formation were used as indicative of positive response to these tests.<sup>21</sup>

#### Anti-inflammatory assay

The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate-buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations of *E. radiata* and *J. verrucosa* extracts so that final concentrations become (0.33, 0.63, 1.25, 2.5, 5, and 10) mg/mL. A similar volume of double-distilled water served as the control. Then the mixtures were incubated at 37°C in a Bio-Oxygen Demand (BOD) incubator (Lab line Technologies) for 15 min and then heated at 70°C for 5 min. After cooling, their absorbance values were measured at 660 nm using UV-Visible spectrophotometer (SHIMADZU, UV 1800). Vehicle and Diclofenac were used as blank and reference drug respectively. The Percentage inhibition of protein denaturation was calculated as: Percentage inhibition = (Abs Control – Abs Sample) X 100/ Abs control.<sup>22,23</sup>

#### Antibacterial assay

The antibacterial activity of methanolic extracts of *E. radiata* and *J. verrucosa* was assessed on the six bacterial strains mentioned above according to the guidelines of the Clinical Laboratory Standards Institute with slight modifications<sup>24</sup>. The Minimum Inhibitory Concentration (MIC) of the extracts was determined using the micro-titre plate method as previously described<sup>25</sup>. The complete protocol of the MIC test was found in the M7-T2 publication of the National Committee for Clinical Laboratory Standards<sup>26</sup>. Briefly, 10% dimethyl sulfoxide (DMSO) was used to prepare a stock solution of 10 mg/mL and the extract was dissolved using a sonicator. The 96-well micro-titre plates were aseptically prepared in a horizontal laminar airflow cabinet. In each well, 100 µL of 10% DMSO was added, and then 200 µL of both *E. radiata* and *J. verrucosa* extracts were added in the first wells from which serial dilutions (two-folds) were made to the desired minimum concentration. The bacterial suspension (100 µL) was added to the wells, and the plates were placed on a shaker at 500 rpm for 30 seconds. Soon after shaking, the plates were read at 620 nm wavelength with a multi-well plate reader to obtain the first reading (t = 0 h), a sterile film was used to cover the plates to prevent evaporation then incubated at 37°C for 24 h. After incubation the absorbance reading at the same wavelength was recorded for each plate. Ampicillin was used as negative control at a concentration of 128 µg/mL. All the experiments were performed in triplicates. The lowest concentration of the extracts that inhibited the visible growth of bacteria was recorded as the minimum inhibitory concentration (MIC).

#### Cytotoxicity assay

Cell proliferation was determined by MTT assay following the methods described by<sup>27</sup> with minor modifications. Briefly, the cells were plated in 96-well tissue plates at a density of  $2.0 \times 10^5$  cells per well and treated with various concentrations (31.5- 1000 µg/mL) of methanolic extracts of *E. radiata* and *J. verrucosa* after which they were incubated for 24 h. Just 5 h before the elapse of 24 h, 10 µL of 5 mg/mL MTT solution was added to each well and the plates were further incubated. At the end of the incubation period, the medium was removed from each well and replaced with 50 µL of dimethyl sulfoxide (DMSO) to solubilize the formazan crystals formed from MTT after the conversion by mitochondrial dehydrogenases of viable cells. Gentle stirring in a

gyratory shaker for 10 mins was done to enhance dissolution. Viable cells were determined by the absorbance at 560 nm using a microplate reader. Results of cellular viability were tabulated as mean absorbance of each extract expressed as a percentage of the untreated control and plotted against extract concentration. IC<sub>50</sub> values were calculated as extract concentrations that reduced the absorbance at 560 nm by 50% of the untreated control wells. To exclude background readings, three wells were seeded with untreated cells in which MTT was not added. Standard drug. A stock solution of 5 µM Camptothecin was used as a positive control. Assays were done in triplicate to ensure reproducibility. The percentage inhibitions of cell proliferation were calculated using the following formula:

$$\% \text{ Cytotoxicity} = \frac{A - B}{A} \times 100\%$$

A = absorbance of negative control (untreated cells) and B = absorbance of treated cells.

Concentrations that inhibit cell proliferation by 50% (IC<sub>50</sub>) were calculated using GraphPad Prism version 6.0 (GraphPad Software Inc., San Diego, CA).

#### Statistical analysis

Statistical analyses were carried out with GraphPad Prism version 6.0 (GraphPad Software Inc., San Diego, CA). All values are Mean ± standard error of mean (SEM), n = 3. One-way Analysis of Variance (ANOVA) and Dunnett's t-test was used as the test of significance. P-value < 0.05 was considered as the minimum level of significance.

## Results and Discussion

### Qualitative Phytochemical Analysis

Over the years, seaweed has been of biomedical interest.<sup>28</sup> Some of the medicinal as well as traditional use of seaweed include disease treatment such as cancer and goiter, treatment of wounds such as burns, and rashes, abdominal wall abscesses treatment, and food purposes.<sup>29,30</sup> There are some clinical trials to support therapeutic recommendations for seaweeds (<https://clinicaltrials.gov/ct2/show/NCT01204957>). However, seaweeds are pivotal mineral sources and are low in sodium.<sup>31</sup> They may be useful in heart conditions due to cholesterol reduction and appetite suppression. Alginates extracted from seaweed have been used in wound dressings.<sup>32</sup> In addition to local therapeutic uses of marine macro-algae, phytochemicals, cytotoxicity, anti-microbial and anti-inflammatory effects of the extracts were investigated. The quest for more information on the pharmacological potentials of natural products is on the increase because of their prospective use in the treatment of various chronic and infectious diseases.<sup>33</sup> The methanolic extracts of *E. radiata* and *J. verrucosa* showed the presence of tannins, phenolics, glycosides, and alkaloids. This is similar to a previous study that showed that three marine red seaweeds contained a variety of phytochemicals including alkaloids, flavonoids, triterpenoids, steroids and tannins.<sup>34</sup> Tannins have been found to have antiviral, antibacterial, anti-parasitic effects, anti-inflammatory, antiulcer and antioxidant properties.<sup>35,36</sup> Most of the therapeutic actions of phytochemicals are ascribed to their biologically active polyphenol components, such as flavonoids and phenolic acids, which possess powerful antioxidant activities that prevent cellular damage due to free-radical oxidation reactions.<sup>37</sup> Phenols are readily absorbed through the walls of the intestinal tract and promote anti-inflammatory conditions.<sup>38</sup> Phenolic and polyphenolic compounds, in isolate or associated to vitamins, such as carotenoids, vitamin E, and vitamin C, are reducing agents that protect human body's specific tissues against oxidative stress.<sup>39</sup> In traditional or modern medicine, alkaloids have been found as starting points for drug discovery. Some alkaloids also possess psychotropic (e.g. psilocin) and stimulant activities (e.g. cocaine, caffeine, nicotine, theobromine), and have been used in entheogenic rituals or as recreational drugs. However, most alkaloids have shown pronounced toxicity.<sup>40</sup> Alkaloids interact with elements of neuronal signal transduction, such as ion channels; ion pumps; neurotransmitter receptors; enzymes, which degrade neurotransmitters; and transporters.<sup>41</sup> Cytotoxic alkaloids often interfere with DNA (via alkylation or intercalation) and microtubules and induce apoptosis.<sup>42</sup> The extracts of *E. radiata* and *J. verrucosa* may

therefore serve as a pool from which lead compounds for drug discovery purposes can be generated.

The results of phytochemicals screening of the methanolic extracts of both *E. radiata* (ER) and *J. verrucosa* (JV) revealed the presence of tannins, phenolic, glycosides, and alkaloids while flavonoids, saponins, terpenoids and phytosterols are absent in both extracts (Table 1).

### In vitro anti-inflammatory activity

Inflammation is a complex biological response to harmful stimuli. This vascular tissue response is directly correlated with pain. The outcome includes elevated denatured protein, vascular permeability, and membrane alteration.<sup>43</sup> Agents that can protect cell membrane against injury are crucial in the event of inhibiting the progression of inflammation. The methanolic extracts of both *E. radiata* and *J. verrucosa* activities suggests good anti-inflammatory activity with a linear response using albumin denaturation method. Although it was suggested that agents that can inhibit protein denaturation can in turn inhibit inflammatory activity,<sup>44</sup> this result does not confirm the anti-inflammatory activities of the extracts as more than one model or anti-inflammatory assays are needed to ascertain this claim.

The *in vitro* anti-inflammatory result (Table 2) of the methanolic extract of *E. radiata* showed mean inhibition of protein denaturation of 13.11, 35.46, 63.9, 79.76, 85.23, and 88.96% for doses 0.33, 0.63, 1.25, 2.5, 5, and 10 mg/mL respectively, while for the methanolic extract of *J. verrucosa*, the result showed mean inhibition of protein denaturation of 1.47, 11.73, 18.48, 21.45, 53.59, and 94.34% for doses 0.33, 0.63, 1.25, 2.5, 5, and 10 mg/mL (Table 2). The methanolic extracts of both *E. radiata* and *J. verrucosa* activities showed good anti-inflammatory activity with a linear response. Maximum inhibition of 88.96 ± 0.69% was observed at 10 mg/mL for *E. radiata* while for *J. verrucosa* maximum inhibition of 94.34 ± 1.48% at 19 mg/mL and standard anti-inflammatory drug (Diclofenac) showed the maximum inhibition, 86.1 ± 1.05 and 80.1 ± 0.70% respectively for *E. radiata* and *J. verrucosa* at 5 mg/mL. The ability of both extracts to inhibit the thermal denaturation of protein was however found to be statistically significant (*p* < 0.05).

### Antibacterial effect of seaweed extracts on selected bacteria

Antibacterial activity is the most important characteristic in fighting infectious disease, providing adequate protection against microbes, biological fluids, and aerosols, as well as disease transmission.<sup>45</sup>

**Table 1:** Phytochemical composition of methanol extracts of *Ecklonia radiata* and *Jania verrucosa*

S/N	Phytoconstituents	<i>Ecklonia radiata</i> (ER)	<i>Jania verrucosa</i> (JV)
1	Tannins	+	+
2	Flavonoids	-	-
3	Phenolics	+	+
4	Saponins	-	-
5	Glycosides	+	+
6	Alkaloids	+	+
7	Terpenoids	-	-
8	Phytosterols	-	-

+: present; ++: highly present; -: absent, ER: *Ecklonia radiata* and JV: *Jania verrucosa*

Antimicrobial properties of medicinal plants are being increasingly reported for proper documentation and further use. In the study, the methanolic extracts obtained from *E. radiata* and *J. verrucosa* show strong activity against most of the tested bacterial strains. The results were compared with standard antibiotic drug. The activities of these extracts against tested pathogens might be connected to the presence of the plant's secondary metabolites.<sup>46</sup> Recent findings suggest that agents with antimicrobial activities can promote cancer apoptosis, inhibit cancer growth and prevent cancer metastasis.<sup>47,48</sup> For these reasons, antimicrobial agents are increasingly being used to assist in the

treatment of cancers.<sup>49</sup> The antibacterial activities recorded against five out of the six tested clinical bacterial isolates is in accordance with several studies on seaweed with good antibacterial activity on most multi-drug resistance clinical bacterial isolates.<sup>50</sup>

**Table 2:** Protein denaturation activity of methanol extracts of *E. radiata* and *J. verrucosa*

Treatment (mg/mL)	<i>E. radiata</i> (%)	<i>J. Verrucosa</i> (%)
0.00	1.00 ± 0.20	1.20 ± 0.12
Diclofenac	86.10 ± 1.05	80.10 ± 0.70
0.33	13.11 ± 0.90**	1.47 ± 1.28**
0.63	35.46 ± 1.20**	11.73 ± 1.70**
1.25	63.90 ± 1.34**	18.48 ± 1.66**
2.5	79.76 ± 0.99**	21.45 ± 0.70**
5	85.23 ± 1.58**	53.59 ± 1.97**
10	88.96 ± 0.69**	94.34 ± 1.48**

All values are Mean ± SEM, n = 3. One-way Analysis of Variance (ANOVA). Dunnett's One-way was used as the test of significance. \*\*p < 0.05 P-value compared with the standard group.

**Table 3:** The Minimum inhibitory concentration (of methanol extracts of *Eklonia radiata* and *Jania verrucosa* on selected micro-organisms

Test organism	MIC (mg/mL)		
	<i>E. radiata</i>	<i>J. verrucosa</i>	Ampicillin
MRSA	0.16	0.78	0.30
<i>Staphylococcus aureus</i>	1.25	0.16	0.16
<i>Pseudomonas aeruginosa</i>	> 10.00	> 10.00	1.20
<i>E. coli</i>	0.31	0.31	0.04
<i>Klebsiella pneumoniae</i>	0.63	0.16	0.63
<i>Streptococcus pyrogenes</i>	1.25	0.63	0.04

MRSA: Methicillin-resistant *Staphylococcus aureus*, *E. coli*: *Escherichia coli*

Taken together with the presence of different phytochemicals the extracts may be hypothesized to possess valuable therapeutic index subject to bioactive isolation and further analysis. The extracts showed

antibacterial activity against the tested bacterial strains. The extracts of *E. radiata* and *J. verrucosa* showed broad spectrum of action as they inhibited the growth of MRSA, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli*, *Klebsiella pneumoniae*, and *Streptococcus pyrogenes* with MIC ranging from 160-10000 µg/mL (Table 3). The highest MIC value was observed in the case of *Pseudomonas aeruginosa* (> 10 mg/mL), which was the least inactivated bacteria specie by *E. radiata* and *J. verrucosa* methanolic extracts. With the lowest MIC value of 160 µg/mL, MRSA was the most inactivated bacteria specie by *E. radiata* methanolic extract while *Staphylococcus aureus* and *Klebsiella pneumoniae* were the most inactivated bacteria specie by *J. verrucosa* methanolic extract. Interestingly, both *E. radiata* and *J. verrucosa* methanolic extracts inhibited the growth of *E. coli* at the MIC of 310 mg/mL. These findings divulge the remarkable growth inhibition of *E. radiata* and *J. verrucosa* against the selected bacteria species.

#### Cytotoxicity activity

The continuous search for novel anticancer agents in medicinal plant is a realistic and promising strategy for cancer management.<sup>51</sup> *J. verrucosa* and *E. radiata* methanolic extract were evaluated as new anticancer agent. Interestingly, the extracts exhibited selective toxicity among the tested cell lines. Briefly, both extracts were potent against the three cancer cell lines relative to the normal cell line and when compared to the known plant-derived anticancer drug camptothecin at low IC<sub>50</sub> values. Several studies have reported the cytotoxicity activity of different types of seaweed extracts against different cancer cell lines.<sup>52-57</sup> This anticancer activity exhibited by both extracts may be attributed to various phytochemical constituents present in the plant. The SI is a measure of impending efficacy against the antagonistic effects of plant extracts.<sup>58</sup> Generally, a higher SI value of a plant extract often implies that the biological activities are potentially not due to metabolic toxins.<sup>59</sup> When the SI value is >1, it is considered that the anticancer compounds are likely different from the toxic compounds or probably the extract is more toxic to the cancer cells than mammalian cells.<sup>60</sup> It is equally pivotal to note that *in vivo* efficacy and toxicity of extracts upon administration do not automatically reflect *in vitro* properties due to other pharmacokinetic and pharmacodynamics factors.<sup>61</sup> The results suggest the use of *E. radiata* and *J. verrucosa* methanolic extract in preparing recipes for cancer-related ailments. The results of the *in-vitro* anticancer activity using MTT assay on KMST-6, HepG-2, MDA-MD-231, and CaCo-2 cell lines are presented in Table 4. The results revealed that the extracts were not toxic to normal cell line (KMST-6) but showed potent cytotoxic activity against cancer cell lines (HepG-2, MDA, and CaCo-2) at specific concentrations when compared to camptothecin. The result showed that an increase in extracts concentration led to a gradual decrease in cell viability as higher concentrations were found to be cytotoxic against the studied cell lines. For the normal cell line (KMST-6), the two extracts showed no cytotoxicity.

**Table 4:** Selectivity index of the plant extracts and camptothecin on different cell lines

Cell line	<i>E. radiata</i>	SI	STD	<i>J. verrucosa</i>	STD	SI
	IC <sub>50</sub> (µg/ml)	( <i>E. radiata</i> )	IC <sub>50</sub> (µg/ml)	IC <sub>50</sub> (µg/ml)	IC <sub>50</sub> (µg/ml)	( <i>J. verrucosa</i> )
CaCo-2	284 ± 0.9	2.1	228 ± 0.6	298 ± 1.7	300 ± 1.5	2.0
HepG-2	230 ± 1.3	2.6	260 ± 1.4	250 ± 0.8	320 ± 1.9	2.4
MDA-MD 231	200 ± 2.0	3.0	210 ± 0.7	220 ± 0.5	290 ± 0.7	2.8
KMST-6	600 ± 1.0	1.0	400 ± 1.8	620 ± 0.8	450 ± 0.9	1.0

Note: SI: Selectivity Index. IC<sub>50</sub>- Concentration of the extract required to inhibit cell growth by 50% as obtained by MTT assay. Selectivity index (SI) indicates differential cytotoxicity of a compound (SI = IC<sub>50</sub> treated normal cells/IC<sub>50</sub> treated cancer cell lines). STD: Standard drug (5 µM Camptothecin).

To ensure the effectiveness of the extracts, selectivity index (SI) was determined for each plant extracts using the IC<sub>50</sub> for the entire tested cell lines (Table 4). *J. verrucosa* and *E. radiata* had an SI value  $\geq 1$  for all of the screened cell lines implying that the cytotoxic activities of these compounds are not by-products of metabolism.

## Conclusion

The biological activities observed in this study serve to support existing scientific evidence to support the use of traditional/ medicinal plants for various uses. The cytotoxicity observation provides preliminary data exposing *E. radiata* and *J. verrucosa* methanolic extracts to have potent cytotoxic activity against HepG-2, MDA-MD-231, and CaCo-2 cells. This requires further studies on the bioactive components for proper assessment of their chemotherapeutic properties, mechanism of cell death, and possible development of therapeutic agents.

## Conflict of Interest

The authors declare no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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