1 MICROBIAL PRODUCTION OF ELLAGIC ACID FROM MANGO PULP
2 PROCESSING WASTE

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Abstract

Ellagic acid has gained momentum recently due to its various properties like anti-mutagenic, anti-carcinogenic, anti-oxidant, and anti-viral and many other benefits to human health. The present study focused on the microbial production of ellagic acid from mango pulp processing industrial waste an alternate method for conventional chemical extraction. Our experiments demonstrated that the 100 µg/ml of ellagic acid was produced by *Micrococcus luteus* from 9% of mango pulp waste and the optimization of ellagic acid production with Pontecorvo medium supplemented with 5.0 g of ellagitannin has yielded 37.80 ± 0.30 mg/g at pH 5.0, temperature 30 °C, ammonium nitrate (nitrogen source), glucose (carbon source), with 1.5% of inoculums after 24 h of incubation. Ellagic acid synthesized was further confirmed with the standard ellagic acid. Applications like drought resistant in plants, anti-microbial activity, anti-parasitic activity and anti-cancer activities have been proven. Ellagic acid exhibited potential applications and further research in product development is promising.

Keywords:

Ellagic acid; Mango pulp waste; Drought in plants; Anti-parasitic; Anti-cancer.

44 **1. Introduction**

45 Ellagic acid (EA) is hydrolysable tannin present in the majority of the
 46 plants and synthesized enzymatically from ellagitannins (ETs) [1] (Figure 7). EAs
 47 have wide applications such as anti-oxidant, anti-tumoural, anti-viral, anti-
 48 microbial, anti-inflammatory, and anti-carcinogenic activities such as anti-
 49 hepatotoxic, anti-cholesteric, anti-fibrogenic and anti-hepatocarcinogenic
 50 properties [2]. EA acts as a remarkable de-pigmentation, gas protective agent and
 51 reduces birth defects. Ellagic acid reacts in the human body that may promote
 52 good health and effective in the prevention of cancer, heart diseases and other
 53 chronic diseases. Nowadays, the demand for natural ellagic acid is increasing due
 54 to their use in the functional food industries as well as in pharmaceutical
 55 industries. Ellagitannins and ellagic acid interact with the cell walls or sites to
 56 facilitate to form complex protein, which prevents the formation of tumours by
 57 preventing and proliferation of metastatic cells [3, 4].

58 It has been chemically synthesised for more than two decades. However,
 59 chemical synthesis involves harsh treatments that are highly aggressive and leads
 60 to corrosion of vessels [5]. EA is being extracted by solvent extraction with
 61 concentrated H₂SO₄ or HCl in the industrial scale [6]. Further, recovering EA
 62 directly from plants would increase production cost as well as loss of biodiversity
 63 [7]. Alternatively, tannin rich mango pulp processing industrial waste can be used
 64 as substrate for bio-conversion into ellagic acid. Hence, the present work is aimed
 65 at producing ellagic acid from ellagitannins by the microbial bio-conversion [8].

66

67 Agricultural residues are the main effective source of natural bioactive
68 compounds [9]. Mango wastes are used by the microbes for their own metabolism
69 and finally produce some bioactive compounds that are important for soil health,
70 plant growth and overall eco-balance. The utilization of agriculture wastes as
71 abundant, bio-renewable and low-cost sources for the production of high value-
72 added products are still under investigation, with limited outcomes. Researches in
73 this field of therapeutic molecules extracted from agro-industrial waste sound
74 good due to their eco-friendly and improve economy of agri-business.

75 Many researchers have already been studied on potential sources of
76 bioactive compounds from agricultural by-products. The food industry is facing a
77 great challenge to handle large quantity of waste generated from product recovery.
78 Billions of dollars are spent on the treatment of agricultural and food waste to
79 recover from the risk caused by these wastes to the environment. Therefore, the
80 risks can be reduced and the costs for treatment of waste can also be reduced.
81 Value for agricultural and product development would be possible through this
82 bioconversion. Mango peels, mango seeds and other wastes are discarded in the
83 industrial process of pulp recovery [10].

84 Mango peel can be used as a valuable, economic and cheaper source for
85 the commercial production of the ellagic acid [11]. Ellagic acid can be obtained
86 by the enzymatic hydrolyzes of ellagitannins. Therefore, the aim of this study was
87 to elucidate the bacterial bio-conversion of ellagitannins into ellagic acid by
88 submerged fermentation. Earlier many reports had studied the role of
89 ellagitannase on ellagitannins biodegradation. Microbial conversion of ellagic

acid from pomegranate husk had shown 65.7 mg/g [8]. Therefore, the aim of this study was to elucidate the bacterial bio-conversion of mango ellagitannins into ellagic acid using submerged fermentation.

2. Materials and methods

2.1. Collection of samples

The mango pulp wastes were collected from Adhiyamaan Mango pulp processing industry, Krishnagiri District, Tamil Nadu. The samples were dried, meshed and used for microbial conversion of ellagic acid.

2.2. Isolation and screening of ellagitannase producer

The soil samples were serially diluted up to 10^{-6} dilutions. The diluted samples were spread plated on to nutrient agar (NA) plates supplemented with 2% of ellagitannin of mango pulp processing waste and was incubated at 37 °C for 24h. After incubation, the bacterial colonies that were able to produce halo-zone around the colonies treated as an ellagitannase positive colonies. Ellagitannase producers were screened with the help of methyl red (0.01 M methyl red in 0.01 N HCl) as pH indicator and flooded on the plates were kept for 5–10 min. Ellagitannase producers that showed red hallow zone around the colony were recorded as positive.

2.3. Production of ellagic acid

The ellagic acid producers were screened based on the diameter of the zone formed on the minimal salt medium (MSM) supplemented with different concentrations (1, 3, 5, 7, 9 and 10%) of mango pulp processing waste as

113 substrate. Ellagic acid production was done using submerged fermentation with
114 5% of inoculums, and this setup was maintained at 37 °C, pH 7.0 for 24 h.

115 **2.4. Optimization and production of ellagic acid**

116 Erlenmeyer flasks (250 ml) containing 100 ml of Pontecorvo medium with
117 different concentration of mango pulp wastes (0.05, 0.07, 0.10, 0.13 and 0.15 g)
118 powder, carbon (sucrose, fructose, glucose, maltose, and xylose) (0.5 g) and
119 nitrogen (peptone, yeast extract, beef extract, ammonium chloride and ammonium
120 nitrate) (0.5 g) source were used for EA production by submerged fermentation of
121 mango pulp waste with the microbial culture. A set up containing no substrate,
122 carbon and nitrogen source has been treated as control. The submerged
123 fermentation was carried out at various temperatures 25, 30, 35, 40 and 45 °C, for
124 various incubation periods like 12, 24, 36, 48 and 72 h under constant 200 rpm
125 agitation with various pH 4.0, 5.0, 6.0, 7.0 and 8.0 with the inoculums sizes of
126 0.1, 0.5, 1.0, 1.5 and 2.0%. The optimized condition was used for the production
127 of ellagic acid from mango pulp waste powder using submerged fermentation.

128 **2.5. Extraction and characterization of ellagic acid**

129 Fermented broth containing ellagic acid was transferred to centrifuge
130 tubes, and ellagic acid was separated by centrifugation at 2000 rpm for 20
131 minutes. The supernatant was transferred to separating funnel, and the equal
132 volume of ethyl acetate was added to the supernatant and mixed well for
133 separation. The upper aqueous layer containing ellagic acid was collected in a
134 beaker and allowed for evaporation of ethyl acetate. About 2 ml of methanol was

added after evaporation and stored in a refrigerator for further experimental analysis using Fourier transform infrared spectroscopy (FTIR).

2.6. Applications of ellagic acid

2.6.1. Stress tolerance in plant

A pot experiment was conducted to investigate the physiological changes in *Vigna radiata* due to the exogenous application NaCl and protective effect of EA. Seeds were treated separately by soaking them for 6 h in a solution containing different concentrations of EA (0, 50, 100 and 200 µg/ml). Pre-treated seeds were sown in pots. Different concentrations of salts (0, 60 and 120 mM) were sprayed on the plants after a week of seed germination and continued for 4 weeks. Plant materials were collected after completion of experimental duration, and assessed for various parameters such as shoot length (cm), root length (cm), shoot fresh weight (g), root fresh weight (g), as well as a physiological parameter like chlorophyll content was measured.

2.6.2. Estimation of chlorophyll

Chlorophyll contents were determined according to the method [12]. The fresh 1g leaves were homogenized in 20–40 ml in 80% acetone in a mortar and pestle. The homogenate was centrifuged at 3000 rpm for 30 min. The supernatant was collected, and the total volume was measured. About 5 ml of the properly diluted extract was taken to measure the absorbance at 645 nm, 663 nm (acetone blank) wavelength using a UV–vis spectrophotometer.

$$\text{Chlorophyll a} = 21.7(A_{663}) - 2.69(A_{645})$$

$$\text{Chlorophyll b} = 22.9(A_{645}) - 4.68(A_{663})$$

158 Total chlorophyll = $20.2(A_{645}) + 8.02(A_{663})$

159 **2.6.3. Antimicrobial activity**

160 The antibacterial activity of the ellagic acid was performed by the agar well
161 diffusion method. Muller Hinton agar medium was inoculated with 0.1 ml of a
162 fresh overnight culture of the *E. coli*, *Bacillus* sp., *Klebsiella pneumonia* and
163 *Staphylococcus aureus* for testing the property of EA antibacterial test and the
164 property of EA. Wells of 6 mm in diameter were punched in the agar and filled
165 with different concentrations (100, 50, 25, 12.5 and 6.75 µg) of the EA. The plates
166 were incubated at 37 °C for 24 h. Then, they were examined for the zone of
167 inhibition, and the results were recorded. The anti-bacterial activity of ellagic acid
168 was measured based on the diameter of the zone of inhibition.

169 **2.6.4. Anti parasitic properties-Larval toxicity test**

170 A laboratory-reared larva was used for the evaluation of the larvicidal
171 activity of ellagic acid. Twenty individuals of first, second, third and fourth instars
172 larvae were kept in a 100 ml glass beaker containing 49 ml of de-chlorinated
173 water and various concentrations of ellagic acid in different concentrations (50,
174 100 and 200 µg) were added. Larval food was given for the test larvae. The
175 control was set up by 50 ml of de-chlorinated water alone. The control mortalities
176 were corrected by using Abbott's formula [13].

177 **Corrected mortality**

178 = $\frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \times 100$
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180
181 **Percentage mortality** = $\frac{\text{Number of dead larvae} \times 100}{\text{Number of larvae introduced}}$
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184 **2.6.5. Anti-cancer activity**

185 The anti-cancer activity was analyzed with various cell lines (MCF-7,
186 HT29, A549, MDA-MB-231 and PA1). The 3-(4,5-dimethylthiazol-2-yl)-2,5-
187 dimethyltetrazolium bromide (MTT) assay, based on the conversion of yellow
188 tetrazolium salt-MTT to purple-formazan crystals by metabolically active cells,
189 provides a quantitative determination of viable cells (Morgan 1998). MCF-7, HT-
190 29, A549, PA1 and MDA-MB-231 the human cancer cells obtained from the
191 Korean cell line bank Seoul, Korea. Cells were plated onto 96-well plates at a cell
192 density of $2 \times 10^{-5} \text{ ml}^{-1}$ per well in 100 μl of RPMI 1640 and allowed to grow in
193 a CO₂ incubator for 24h (37 °C, 5% CO₂). The medium then removed and
194 replaced by fresh medium containing different concentration of sample for 24 h.
195 The cells were incubated for 24–48 h (37 °C, 5% CO₂). Then 20 μl MTT stock
196 solutions (5 mg/ml in PBS) was added to each well and incubated for 5 h. The
197 medium was removed and 200 μl DMSO added to each well to let dissolve MTT
198 metabolic product. Then the plate shaken at 150 rpm for 5 min, and the optical
199 density was measured at 620 and 570 nm. Untreated cells (basal) were used as a
200 control of viability (100%), and the cell viability was calculated as follows:

$$201 \quad (\text{A570-A620, Sample/A570-A620nm, control}) \times 100$$

202

203 **3. Results**

204 **3.1. Isolation and screening of ellagic acid producing bacteria**

205 The soil samples were collected from 8 different site (Garden site GS1,
206 GS2, Periyar University PU3, PU4, PU5, agriculture field AG6, AG7, AG8), and

nearly 200 different colonies were isolated by standard plate count method. The number of colonies was counted for each sample at 10^{-6} dilutions. Nearly 15 bacterial isolates were shown positive for tannase production, of which one isolate PU4 was able to produce a 15 mm wider zone than other strains. Hence, the bacterial culture PU4 has been chosen for further experiment analysis.

3.2. Production of ellagic acid

Ellagic acid production was accomplished by inoculating MSM medium substituted with different concentrations of mango pulp processing waste (1%, 3%, 5%, 7%, 9%, and 10%) at 37 °C for 24 h. After fermentation, the cell growth was observed at 254 nm (OD) for ellagic acid production (Figure 2). The concentration of mango process was identified as 9% the production of ellagic acid taken for further scale-up production and extraction. It was calculated from the standard graph that 100 µg/ml of ellagic acid was produced when 9% of mango pulp was used as a substrate.

3.3. Optimization of ellagic acid

To optimize the media for maximum production of EA components such as different carbon and nitrogen sources were optimized. Even various temperatures, pH, incubation period and inoculums concentration were maintained. Among several carbon and nitrogen sources used for the EA production, glucose had yielded 24.68 mg/g and ammonium nitrate 24.52 mg/g of EA. Among various temperatures used for the EA production, 30 °C showed a maximum (24.38 mg/g) of EA production. Among various pH used for the EA production, pH 7.0 showed a maximum 24.51 mg/g, in case of incubation period

at 24 h, the EA production is 24.37 mg/g and the size of the inoculums (1.5%) production of EA is 24.5 mg/g. The production of EA after optimization was about 37.80 ± 0.30 mg/g that means a 1.3-fold increase in production.

3.4. Characterization of ellagic acid

Characterization of the ellagic acid was done by using FTIR by their functional groups. The test compound was analyzed by FTIR, the results of the spectrogram represent the presence of glycosidic groups $\nu_{(O-H)}$ that is the presence of OH stretching with a broad peak at 3554 cm^{-1} . The carboxylic substituent's C=O stretching peak at 1691.57 cm^{-1} . The amide group at $1193\text{--}1057\text{ cm}^{-1}$ and the alkaloids at 638.44 cm^{-1} correspond to ellagic acid. All these bands are more or less similar to the standard ellagic acid (Figure 1). Based on the functional group and the chemical structure of the extracted compound, it may be presumed as ellagic acid. The sample was further confirmed with the comparison with standard ellagic acid.

3.5. Applications of ellagic acid

3.5.1. Stress tolerance in plant

Salt stress severely affects *Vigna radiata* (green gram) varieties although the effects of NaCl on the shoot and root length varied among green gram varieties (Figure 3). Salt stress had a diminished shoot fresh and dry weight as well as root fresh and dry weight. Treating seeds with EA had a pronounced effect on the above parameters, while the control plant was severely affected by salt treatment. The highest concentration of $200\mu\text{g/ml}$ EA reduced salinity effects of NaCl treatments in plants, resulting in longer shoots when compared to other

253 concentrations of NaCl. Whereas medium concentrations of 55µg/ml EA had no
254 effect in plants, and the concentration of NaCl was maintained. Root length was
255 generally variable in all salt treatments and little influenced by the addition of EA
256 in *Vigna radiata*.

257 **3.5.2. Chlorophyll content of plant**

258 Application of EA evaluated the effect of salinity and enhanced the growth
259 of the plants. Among different concentrations of EA, 200µg/ml had increased the
260 chlorophyll content of leaf under saline conditions at *Vigna radiata* (green gram)
261 at 120 mM of salinity compared with others. With regard to chlorophyll b, 200
262 µg/ml of EA increased the content in *Vigna radiata* (green gram) at 120 mM NaCl
263 but not at 60 mM NaCl, while in *Vigna radiata* (green gram), 200 µg of EA was
264 more effective at both salinity treatments. Salinity stress also reduced the total
265 chlorophyll amounts and the application of EA alleviated the effect of salinity
266 when applied at 200 µg/ml at *Vigna radiata* (green gram), an increase of the total
267 chlorophyll was also observed after EA addition, particularly at high
268 concentration while (Figure 4).

269 **3.5.3. Anti-microbial activity**

270 The antimicrobial activity of the ellagic acid was assessed by testing the
271 organism for antimicrobial sensitivity, and the pathogen was found to be resistant
272 to the drug used. The antimicrobial activity of the extract increased linearly with
273 the increase in the concentration of extract (µg/ml). As compared with standard
274 drugs, the result revealed that in the extract the bacterial activity for

275 *Staphylococcus aureus* (23 mm) and *Bacillus* sp. (25 mm) was sensitive as
276 compared to *E. coli* and *Klebsiella pneumonia* (Results not shown).

277 **3.5.4. Larvicidal activity**

278 The larvicidal activity of ellagic acid was tested with mosquito larvae
279 (*Aedes aegypti*). It was observed that 15% mortality was noted on the 2nd day
280 after treatment with 50 µg of EA while it was 50% mortality with 200 µg EA. The
281 mortality was 100% after 4th day and 5th-day treatment with 200 µg and 100 µg,
282 respectively. In comparison to standard ellagic acid (100 µg), which gave 100%
283 mortality on the third day, it was observed that ellagic acid produced from mango
284 pulp waste gave 100% mortality on the 5th day when 100 µg was used (Figure 5).

285 **3.5.5. Anti-cancer activity**

286 Ellagic acid was screened using a cell viability assay (MTT assay) to
287 examine the anti-cancer activity with various cell lines such as MCF-7-Breast
288 cancer cell line, HT-29-Colon Cancer cell line, A549 – Lung Cancer cell line, PA-
289 1 – Ovarian Cancer cell line, MDA-MB-231 – Breast Cancer cell line for 72 h.
290 Cell lines were treated with different doses of ellagic acid and thymoquinone for
291 72 h, and then anticancer efficacy was observed for each cell line.

292 **PA-1- Ovarian Cancer Cell line**

293 The IC₅₀ value for this cell line was 18.74µg/ml, which is between the
294 therapeutic index of 17.82–19.71 µg/ml and this was compared with
295 thymoquinone (16.11 µg/ml) (Figure 6).

296

297

298 4. Discussion

299 The bioactive compounds are rich in plants and used both in traditional
300 and modern medicine against various ailments. However, bioactive compounds
301 available in many plants are not fully utilized and remains as an unexplored area.
302 Ellagic acid is a polyphenol, in particular, an ellagitannin, which can be found in a
303 great proportion in many plants, has been known for its health benefits many
304 years ago. Researchers have started work on pharmaceutical applications of
305 ellagic acid only in recent years [14]. The phenolic nature of ellagic acid has the
306 capacity to form complexes with other molecules and influence positive health
307 benefits. Anticancer property against breast cancer, prostate cancer and colon
308 cancer had been known for many years [15].

309 Bioavailability of ellagic acid has become as a great challenge, and it has
310 become a hurdle for in vitro studies and in vivo studies [16]. There is an urgent
311 need for the formulation of a novel method for the production of ellagic acid using
312 microbial sources using the cheaper resource as a substrate. Ellagitannins of
313 mango chosen for ellagic acid production based on earlier reports stated that
314 strawberry, cranberry, blueberry and blackberry are a rich source of ellagitannin.
315 Similarly, have attempted on the ellagic acid production from the aqueous extract
316 obtained from pomegranate husk (*Punica granatum*) and from acron fringe oak
317 [17]. Many similar other agro wastes can be used for the production of ellagic
318 acid which is rich in ellagitannins and mango pulp processed waste was used for
319 this study.

320 Similarly, about 65.7 mg/g of ellagic acid produced with 7 % of
 321 pomegranate husk was the substrate [18]. Pomegranate has more amount of
 322 ellagitannin than mango pulp. Yet another study has used *A. niger* for the
 323 production of ellagic acid was able to produce only 21.19 mg/g with 7.5 g of
 324 pomegranate husk [19]. The amount of ellagic acid produced was low due to the
 325 tannin content of mango pulp waste also less. The previous had achieved 23.1%
 326 of ellagic acid from 12g of Cresetoe bush at 36 h using *A. niger* GH1 strain [17,
 327 20]. *A. niger* produces tannin acyl hydrolase which are able hydrolyzes
 328 ellagitannin-rich plant materials [18, 21]. Similar studies had been conducted on
 329 the tanning degrading abilities of fungal strains GH1 and PSH [22].

330 Previous studies also have achieved 0.256 mg/l of ellagic acid with a
 331 temperature 35 °C using mahua bark and *Aspergillus awamori*. Some researchers
 332 reported that the rising temperature would affect the solubility of the protein, so
 333 that enzyme activity increased. But higher temperatures would denature the
 334 enzymes thus decreased the activity [23]. By using different ranges of all
 335 parameters in response surface methodology (RSM), the optimized condition for
 336 the high production of ellagic acid. The maximum ellagic acid production was
 337 observed with the following parameters glucose (0.83), ammonium nitrate (0.83),
 338 temperature (38.3 °C), pH (6.6), the incubation period (48 h), potassium chloride
 339 (0.37) and inoculums size (1.83%).

340 Using the optimized conditions, 496.75 mg/g of ellagic acid production
 341 was achieved which was 1.4 times increase in the production. The previous study
 342 had shown that *A. niger* HT4 which degraded the ellagitannins present in the

343 pomegranate had produced 350.21 mg/g of EA [19]. Optimization of EA
344 production using ellagitannin of MPPW as the substrate has yielded 37.80 ± 0.03
345 mg/g EA at 37 °C temperature, pH, carbon, nitrogen source [24]. There has been
346 2.3-fold increased production of ellagic acid (24.68 mg/g) than normal when
347 glucose was used at 10.0 mg/g concentration.

348 The previous study reported that the ellagic acid production was carried
349 out from the ellagitannins of containing the amount of ellagic acid was evaluated
350 by HPLC. HPLC analysis of ellagic acid was displayed at 5.490 min with 254 nm
351 [25]. Prior to the characterization, recovery of ellagic acid was carried out using
352 ethanol formic acid extraction [19]. The characterization of ellagic acid obtained
353 is important for understanding their properties and applications. It was confirmed
354 by the comparison of standard ellagic acid. Ellagic acid is a complex metabolite
355 therefore all the characterization has to carried out to confirm it with the standard
356 molecule.

357 Also previous study reported that among different concentrations of EA,
358 110 µg/ml of EA increased the chlorophyll a under saline conditions at V1 and in
359 V2 at 120 mM of salinity, With regard to chlorophyll b, 55 µg/ml of EA increased
360 the content in V1 at 60 mM NaCl but not at 120 mM NaCl, while in V2, 110 ppm
361 of EA was more effective at both salinity treatments. With regard to chlorophyll
362 b, 200 µg/ml of EA increased the content in *Vigna radiata* (green gram) at 120
363 mM NaCl but not at 60 mM NaCl, while in *Vigna radiata* (green gram), 200 µg of
364 EA was more effective at both salinity treatments [26].

Salinity stress also reduced the total chlorophyll amounts and the application of EA alleviated the effect of salinity when applied at 200 µg/ml at *Vigna radiata* (green gram), an increase of the total chlorophyll. Hence, from the previous study when compared to this study it confers that the ellagic acid produced the mango pulp processed waste has a better activity for the plant growth.

Antibacterial activity of ellagic acid has been studied using *Staphylococcus epidermatis*, *B. cereus*, *K. pneumonia*, and *S. typhi*. The results proven that ellagic acid produced from the mango waste is potential antimicrobial agent. Similar other study had proven that that the ellagic acid of pomegranate and punicalagins significantly inhibited the growth of pathogenic *E. coli*, and *Pseudomonas aeruginosa* [27]. EA exhibited significant antibacterial activity than gentamycin and streptomycin when they were tested with above bacterial strains [28].

Previous study already reported that anti-larvicidal activity bioassays were also carried out using ellagic acid derivative compounds isolated from the bark of *Elaeocarpus parvifolius*, which showed potent anti-parasitic properties. A similar study was conducted by these compounds was evaluated for the power to inhibit *Babesia gibsoni*. This proves that ellagic acid has the anti-parasitic impact from the previous studies and this study [29]. A similar study was carried out by the larvicidal activities of *Tephrosia egregia* extract and its major component dehydrototenone was studied. High inhibitory activity was found for dehydrototenone and fuel and ester extracts from roots and stems, respectively.

388 Among the tested extracts, the dissolving agent extract from stems showed potent
389 larvicidal activity ($LC_{50} 12.88 \pm 0.64$), whereas low activity was found for de-
390 hydrotrotenone [30].

391 However, the anti-cancer activity showed that the IC_{50} value of the ellagic
392 acid is $46.08 \mu\text{g/ml}$, which is in between the therapeutic index of $43.2254.05$
393 $\mu\text{g/ml}$. So, the sample has better activity for lung cancer (A549). Thymoquinone
394 has an IC_{50} value of $43.90 \mu\text{g/ml}$. Already found that A549 has the IC_{50} value
395 $>60 \mu\text{g/ml}$ [31]. Previous study reported that the A 549 cell line was resistant to
396 anticancer compounds. It was found that IC_{50} value for A549 and RAW264.9
397 cells treated with ellagic acid nanocomposite was 72.3 and $24.8 \mu\text{g/ml}$
398 respectively, [32]. EA is one such compound that has been tested as a
399 chemotherapeutic, radio sensitizer, antioxidant, etc. EA studies are performed on
400 numerous growth varieties in laboratories which exhibit a big anti-carcinogenic
401 activity. In some studies, evaluation is also performed in mice models. EA and
402 radiation might persuade to be an efficient antitumor drug in clinics for the
403 treatment of cancer.

404 The maximum amount of ellagic acid produced from the mango waste
405 supplemented fermentation medium using native bacterial isolate. The ellagic acid
406 produced showed anti-larval, anti-cancer, anti-microbial and stress-tolerant
407 properties are promising drug candidates for future use. The current research data
408 can appeal to smaller industries. Potential molecule like ellagic acid has great
409 impact on the field of medicine, pharmaceutical, agriculture and food industries.

Further, research on the molecular mechanism of various applications of ellagic acid would improve their wider usage.

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517 **Figure caption**

518 **Figure 1:** Characterization of ellagic acid produced by the natural isolates using
519 FTIR

520 **Figure 2:** Effect of different concentration of mango pulp waste on the ellagic
521 acid production

522 **Figure 3:** Drought resistant properties of the ellagic acid on green gram plants:
523 root length (A) and shoot length (B), fresh root (C) and fresh shoot (D) and inter-
524 node (E) *Vigna radiata* of under non-saline (control) and saline (60 mM and 120
525 mM NaCl) conditions.

526 **Figure 4:** Effect of EA on chlorophyll a, chlorophyll b (b) total chlorophyll (TC)
527 (c) of *Vigna radiata* (green gram) under saline and non-saline conditions.

528 **Figure 5:** Larvicidal Activity of ellagic acid at different concentration test against
529 *Aedes aegypti*

530 **Figure 6:** Anticancer activity of ellagic acid at the different concentration tested
531 against PA1 cell lines

532 **Figure 7:** Schematic showing enzymatic hydrolysis of ellagitannin
533

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Fig 2: Effect of different concentration of mango pulp waste on the ellagic acid production

A

B

C

D

E

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