

Allelopathic potential of *Annona muricata* (L.) on physiological and biochemical changes of *Vigna radiata* (L.) and *Eleusine coracana* (L.) Gaertn.

Edwina Kannan¹, Leela Palayian^{2*} 

¹Research Scholar, (Reg. No: 19211172262019) Department of Botany, PG Research Department, Rani Anna Government College for Women, Tirunelveli-8, Tamil Nadu, India (Affiliated to Manonmanium Sundaranar University, Tirunelveli, Tamil Nadu, India).

²Department of Botany, Rani Anna Government College for Women, Tirunelveli, India.

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ABSTRACT

An experiment was conducted to assess the allelopathic potential of leaf extract of *Annona muricata* (L.) on physiological and biochemical changes of *Vigna radiata* (L.) and *Eleusine coracana* (L.) Gaertn. *Annona muricata* inhibits and stimulates other plants' growth, a phenomenon called "allelopathic effect." Crude water extract of the leaves of *A. muricata* was treated on *V. radiata* and *E. coracana* in different concentrations such as 1%, 2%, 3%, 4%, and 5%, respectively. A control crop was treated with distilled water. The morphological and physiological effects on *V. radiata* and *E. coracana* were observed and recorded. Data were recorded on seed germination (after 10 days of seed sowing), growth, and biochemical composition (15 days old seedlings). Reduction in germination percentage of *V. radiata* and *E. coracana* was recorded using leaf extract of *A. muricata*. The outcome of this research work showed that the extracts from the leaves of the *A. muricata* plant inhibit and stimulate the growth and development of the test seedlings. The biochemical constituents of test seedlings, such as chl a, chl b, carotenoid, protein, amino acid, carbohydrate, peroxidase, and catalase were observed. The extracts were absorbed through the roots of these plants, affecting physiological processes such as photosynthetic and transpiratory rates, stomatal closure, and reduced chlorophyll content, resulting in wilting, tissue maceration, and stunted growth. Between the two crops, more allelopathic effect was recorded in *E. coracana*. These results showed that there are differences in allelopathic inhibition and stimulation of crops.

1. INTRODUCTION

Allelopathy is the production of specific biomolecules by plant species (mostly secondary metabolites) that can induce suffering or benefit other plant species around it [1]. Allelopathy is the active or passive release of chemical substances by one plant into the environment by root exudation, leaching, volatilization, and decomposition. Allelochemicals are secondary metabolites and phytochemicals that correspond to the following classes: flavonoid, terpenoid, phenolic compound, organic cyanide, glucosinolate, saponin, alkaloid, and long chain fatty acid structures [2]. These are released as a result

of enzymatic processes or as a result of environmental stress. Allelochemicals' structure and concentration vary depending on biological and non-biological inputs, hence their targets and functions vary [3]. Green plants produce a wide range of compounds that are not involved in the primary metabolism of plants and hence are called secondary products or metabolites. The biosynthetic pathways of the secondary compounds as off-shoots of the primary metabolism elaborated these pathways of allelochemical(s) origin [4,5]. Competition is one of the most common ways of interaction between cultivated crops and their surrounding plants [6,7].

The plant inhibits the growth of other plants growing around it, a phenomenon called allelopathic effect. Allelopathic characteristics can be found in all parts of a plant, from the foliage and flowers to the roots, bark, soil, and mulch. Almost all allelopathic plants, especially during fall, store their protective chemicals in their

*Corresponding Author

Leela P, Department of Botany, Annamalai University, Chidambaram, India. E-mail: leeladde72 @ gmail.com

leaves. These toxins can impact surrounding plants as the leaves fall to the ground and decompose. The allelopathic effect on plant growth and development depends on the compounds involved, their concentration and stability in the given environment, and plant response to the compounds or their microbial byproduct(s). The availability of biologically active concentrations of allelopathic compounds in the soil environment depends on the rates of input, absorption, and adsorption by seeds and roots, fixation in soil organic matter, leaching and microbial degradation, and persistence. In arid environments, the retention of inhibition is greater. Hence, they persist in the environment for a longer time. Allelopathic crops can help reduce noxious weeds and plant pathogens, enhance soil quality, and increase crop production when used as a cover crop, mulch, smother crop, green manure, or cultivated in rotational sequences. As a result, allelochemicals derived from allelopathic crops may be helpful in the creation of biological herbicides and pesticides [8]. Allelopathy has an alternative for developing eco-friendly agricultural practices, enhancing crop productivity, and maintaining ecosystem stability [9].

The tree is the dominant component in the agroforestry system. It impacts the related crops plants through leaf fall, exudates, and compounds produced at various stages of leaf litter decomposition. Inhibitory chemicals can be found in various plants and organs, including leaves, flowers, fruits, and buds. In agroforestry, where many crops are grown below the canopy of trees, allelopathic interactions are implicit. Phytochemicals present in tropical trees allelopathically retard the growth of associated crop species [10]. Coconut (*Cocos nucifera* L.), cashew (*Anacardium occidentale* L.), jackfruit (*Artocarpus heterophyllus* Lam.), mango (*Mangifera indica* L.), tamarind (*Tamarindus indica* L.), and teak (*Tectona grandis* L.f.) are examples of multipurpose trees that are often planted in agroforestry systems.

The allelochemical activity varies depending on the research methodology and operational processes [11]. The allelochemical released into the environment is usually not a single substance, and the amount of allelochemicals released varies depending on the circumstances. When Albuquerque *et al.* [12] investigated the allelopathic potential of plants, the type and amount of allelochemicals released by them were taken into account. In some instances, one allelochemical may not exhibit allelopathic action as a single component, but it may increase allelopathy when combined with additional allelochemicals.

Annona muricata is a coveted tropical tree and a wealth of phytochemical investigations has been conducted for this fruit plant. *Annona muricata* is a good source of bioactive compounds, such as polyphenols, acetogenins, and alkaloids [13,14]. In the *A. muricata* plant, polyphenols and acetogenins have been studied extensively. *Annona muricata* L., also known as soursop, graviola, guanabana, paw-paw, and sirsak, belongs to the Annonaceae family, which has approximately 130 genera and 2,300 species. *Annona muricata* has been shown to have a wide range of biological functions, in addition to being an essential food source and an indigenous medicinal plant. The anticancer, antiparasitic, and insecticidal effects of this plant have been determined to be the most promising in previous investigations. The fruit contains many nutrients such as vitamin C, calcium, dietary fiber, iron,

protein, cholesterol, sodium sugars, unsaturated fats, etc. The fruit improves the immune system and avoids infections in the body.

The production of agricultural crops in Tamil Nadu is still on a subsistence scale mainly due to traditional farming methods which may not be sufficient to cater to the needs of the growing population. In order to boost the present production status on sustainable basis, commercial cultivation with maintaining sustainability in agricultural production is a great challenge. One of the options is to promote and popularize agroforestry through scientific intervention using crop combinations from the indigenous plant resources. However, for selecting suitable crop combinations in agroforestry systems for better productivity, the compatibility among the woody and non-woody components has to be checked thoroughly. The present study, therefore, aims to analyze the inhibitory and stimulatory effects of aqueous extracts of traditional medicinal perennial species of *A. muricata* on seed germination, morphological, physiological, and biochemical activities of *Vigna radiata* and *Eleusine coracana*.

2. MATERIALS AND METHODS

The experiment was carried out in the Botanical garden of Rani Anna Government College for women, Tirunelveli, India. The study site is located at 8.7139°N latitude 77.7567°E longitude. Fresh mature leaves of *A. muricata* plant were collected, washed separately with several changes of sterile distilled water, and air dried for 7 days. They were blended with a sterile blender and sieved with a fine sterile cheese clothes to obtain fine powder. Cold water extract of the blended leaves of *A. muricata* was prepared separately by adding 10, 20, 30, 40, and 50 g of leaves of the powdered test plants separately to the conical flask containing 1,000 ml of sterile distilled water and allowing them to settle for 24 hours. The resulting solutions were filtered through sterile cheese cloth, which then gave the plant extract concentrations of 1%, 2%, 3%, 4%, and 5%, respectively. The plant extracts were put in sterile McCartney bottles and labeled accordingly, while the research lasted. All preparations were carried out in replicates of three each. Distilled water was used as control.

The viable seeds of *V. radiata* and *E. coracana* were sown in mud earthen pots measuring 10 cm in diameter and 10 cm in depth, packed with garden soil. Freshly prepared aqueous leaf concentrates of 1%, 2%, 3%, 4%, and 5% were sprayed on the surface of *V. radiata* and *E. coracana* seedlings to uniformly cover the entire seedling surface. The control pots were sprayed with distilled water. They repeated each procedure three times. After 10 days of seed sowing, germination was recorded. 15 days after sowing, the seedlings were rooted out and washed with tap water to remove any soil left on the roots. For biophysical analysis, the root length and shoot length were measured in centimeter. Biochemical parameters such as chlorophyll a and b (using Arnon's method), carotenoid (using Krik and Allen's method), carbohydrate (using Anthrone's method), protein (using Lowery *et al.*'s method) [15], amino acid (using Moore and Stein's method), and catalase (using Machly and Chance's method) were examined in the seedlings.

2.1. Statistical Analysis

All the experiments were carried out in triplicates and carried out separately thrice with consistent findings. Data are defined

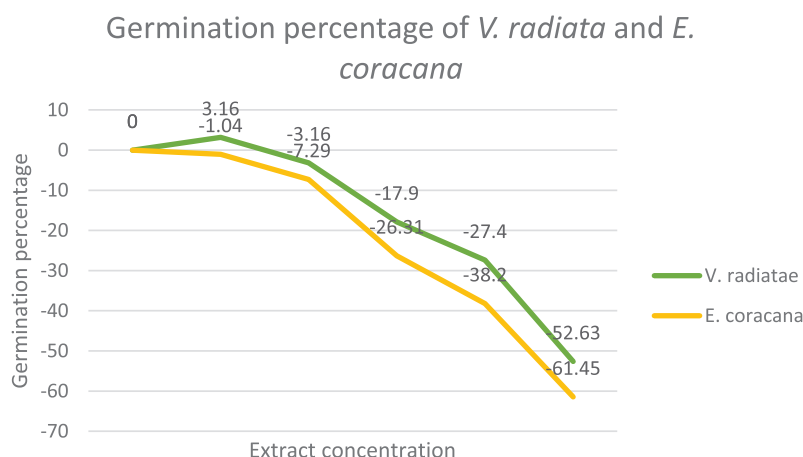


Figure 1: Allelopathic influence of *A. muricata* on the germination percentage of *V. radiata* and *E. coracana* seedlings (10 days after sown).

as mean of replicates \pm SD. Differences between treatments were then tested using one-way analysis of variance for each parameter under review, followed by Duncan's test ($p \leq 0.05$).

3. RESULTS AND DISCUSSION

Significant effects of the treatment on germination percentage were recorded with the interaction between *A. muricata* and annual crops such as *V. radiata* and *E. coracana* (Fig. 1). The germination percentage of *V. radiata* in 1% aqueous leaf extract of *A. muricata* was stimulated (3.16%) and inhibited (1.04%) in *E. coracana* over control. Reduction in germination percentage was noted with application of *A. muricata* leaf extracts of 2%, 3%, 4%, and 5%, respectively, on test crops compared to control. The stimulatory effect was recorded at 1% extract treatment on *V. radiata* only. The stimulatory effect of germination percentage has been attributed to test crops due to the allelochemicals of leaf extracts of tree species. Venkateshwarlu *et al.* [16] recorded 90% inhibition in seed germination of okra when treated with leaf extract of *M. indica*. Both stimulatory and inhibitory effects were noted by Sahoo *et al.* [17] on seed germination of annual crops, with the aqueous leaf leachate of *M. indica*. Inhibition of seed germination by allelochemicals has been attributed to suppressing the mitotic activities of young cells [18]. Inhibition of germination occurred due to the presence of chemicals in the leaf extract of *A. muricata* such as muricoreacina, murihexocina, kaempferol, quercetin 3-O-glucoside, quercetin 3-oneohesperidoside, quercetin 3-O-robinoside, quercetin-O-rutinoside, quercetin 3-O-arhamnosyl, robinetin, tangeretin, and taxifolin. Shahena *et al.* [19] reported that the aqueous extract of *Wedelia* significantly inhibited the growth of pulse seedlings with a more pronounced effect at higher concentrations. This supports our present findings.

When the test plants were treated with different concentrations of aqueous leaf extract of *A. muricata*, they showed reduction in the growth of the seedlings over control (Fig. 2). Only 1% extract treatment in *V. radiata* showed a stimulatory effect of 2.07% root length over control and 2.97% shoot length over control, respectively. The root lengths were more sensitive to allelochemicals than the shoot length, thereby ultimately affecting

the plant growth [20]. The leaf extracts inhibited the seedling growth at different inhibition values. The inhibitory activity was test species, plant parts extract-dependent. In few cases, the growth stimulatory activity was also observed. Many researchers, around the world reported this type of growth inhibition by the aqueous extract of allelopathic plants [21,22].

The tannin from *A. muricata* L. leaves also inhibited the seedling growth of *V. radiata* and *E. coracana*. The inhibition effect is concentration-dependent. This means that the inhibition in the growth of the seedling took effect with increasing concentration. In the control, the root length and shoot length after 15 days of seed sowing of *V. radiata* was 2.41 and 11.29 cm and of *E. coracana* was 1.86 and 10.20 cm, respectively. Negative values were obtained in the treatments indicating that the seedling ceased to grow, which may have led to the impairment in the seedling's metabolic activities, resulting in a decrease in their root and shoot length [23]. Cell division also stopped because no shoots grew in all the treatment. In the study of Arowosgabe *et al.* [24], similar concentrations of 2, 4, 6, 8, and 10 mg/ml were used in observing the allelopathic effect of *Aloe ferox* mill root extract on tomato. Morikawa *et al.* [25], in their study, reported the growth inhibitory activity of *A. muricata*.

Inhibition in the seedling growth of *Echinochloa crus-galli* was observed using the same concentrations. Phenolic compounds, like coumarin, had inhibitory effects on seedling growth and seed germination on both test seedlings. The chemicals involved in allelopathic interactions are present in virtually all plant parts including leaves, stems, fruits, roots, rhizomes, buds, and seeds [26,27].

The results (Figs. 3 and 4) showed that the pigment contents of the test crops are highly influenced by *A. muricata* leaf extracts. In *V. radiata* test seedlings recorded, 1% extract treatment pigments were stimulated (Fig. 3). At 1% extract, stimulations of chlorophyll a (5.56%), chlorophyll b (3.52%), and carotenoid (2.12%), respectively, were compared to control. However, in *E. coracana* (Fig. 4), there was no stimulation noted at 1% extract treatment. Between the two crops, more inhibition was observed in *E. coracana*. The inhibition

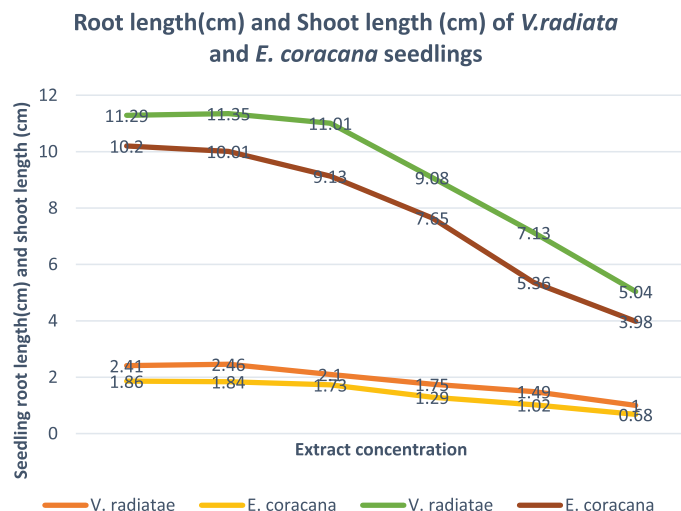


Figure 2: Allelopathic influence of *A. muricata* on root length (cm) and shoot length (cm) of *V. radiata* and *E. coracana* seedlings (15 DAS).

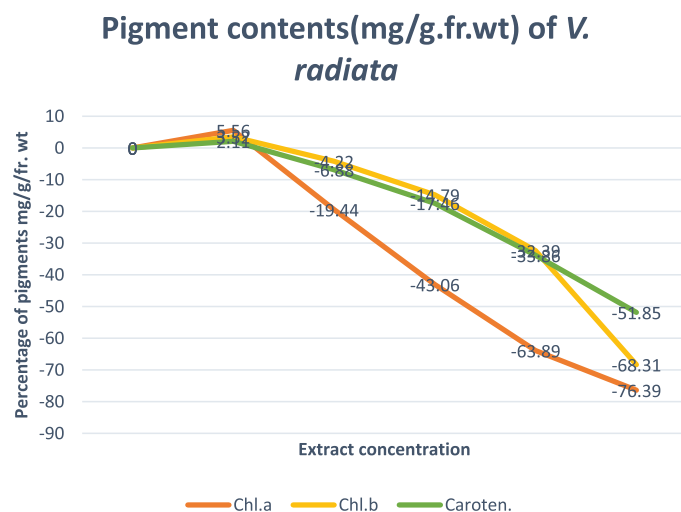


Figure 3: Allelopathic influence of *A. muricata* on Chl a, Chl b, and carotenoid (mg/g fr. wt.) contents of *V. radiata* seedlings (15 DAS).

was concentration-dependent. At 5% extract treatment, more inhibition was recorded in both crops. The inhibition of pigments in *V. radiata*, chlorophyll a (76.39%), chlorophyll b (68.31%), and carotenoid (51.85%), and in *E. coracana*, chlorophyll a (79.57%), chlorophyll b (70.51%), and carotenoid (74.80%), was more than that in the control. The allelochemicals of *A. muricata*, such as muricatacin, isoquinolines, anonaine, anoniin, coreximine, lipids, gentisic acid, lignoceric acid, stearic acid, genistein, glycitein, homoorientin, isoferulic acid, kaempferol, quercetin 3-O-glucoside, quercetin 3-oneohesperidoside, quercetin 3-O-robinoside, quercetin-O-rutinoside, quercetin 3-O-a-rhamnosyl, robinetin, tangeretin, taxifolin, vitexin, caffeic acid, hydroxycinnamic acid, gentisic acid, hydroquinone, gallic acid, tannin, flavonoids, phenolic compounds, etc., might inhibit the pigments of test seedlings. Allelochemicals have several effects on plant photosynthesis, including inhibiting or damaging the synthesis machinery and hastening the breakdown of photosynthetic pigments (Table 1). Photosynthetic pigment contents are reduced, which blocks the energy and electron transfer, reduces adenosine triphosphate (ATP) synthesis enzyme activity, inhibits

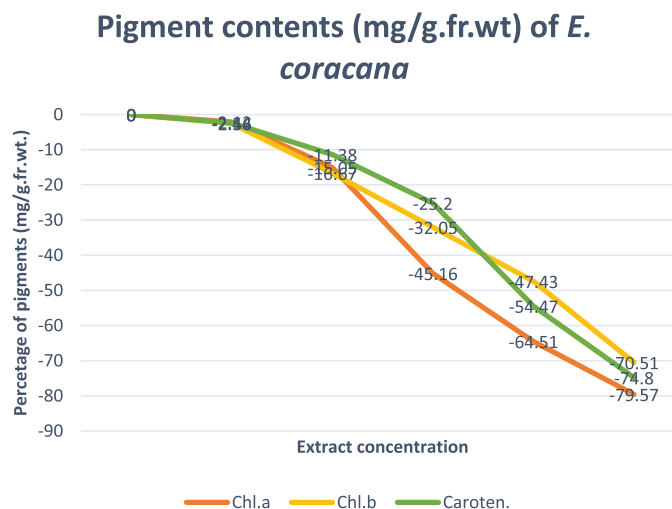


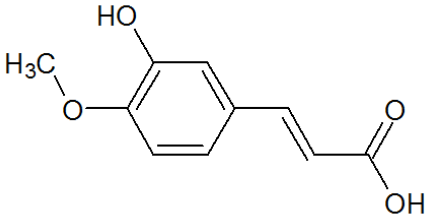
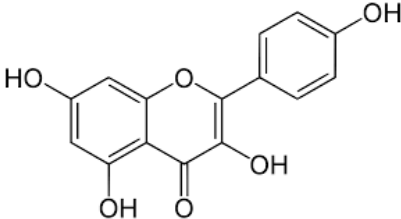
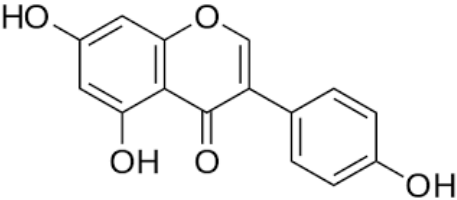
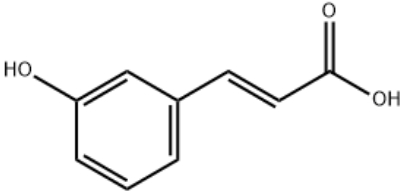
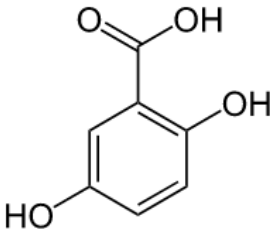
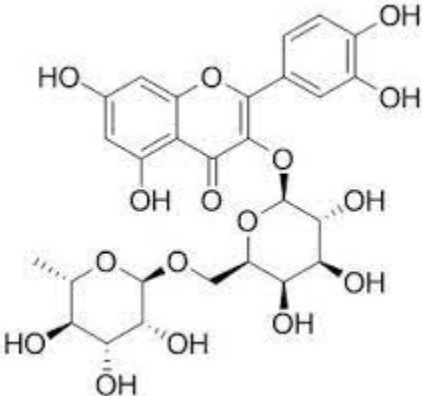
Figure 4: Allelopathic influence of *A. muricata* on Chl a, Chl b, and carotenoid (mg/g fr. wt.) contents of *E. coracana* seedlings (15 DAS).

ATP synthesis, and affects stomatal conductance and transpiration, all of which inhibit the photosynthetic process. The allelochemicals influence photosynthesis primarily via altering PS II function [28–30]. Poonpaiboonpipat *et al.* [31] found that a high concentration of essential oil from lemongrass (*Cymbopogon citratus*) leaves reduced the chlorophyll a and b and carotenoid content of barnyard grass and affected alpha-amylase activity in seeds, implying that essential oils interfere with photosynthetic metabolism.

A decrease in chlorophyll and carotenoid content in response to *A. muricata* allelochemicals indicates that it has a negative impact on photosynthetic efficiency. The observed effects are similar to the effects of essential oils, such as *Eucalyptus citriodora* and *Artemisia scoparia*, which reduce chlorophyll content and thus affect photosynthetic activity [32–34]. Scott [35], in his study, found that chlorophyll a, b, and carotenoid are key pigments of chloroplasts of plants that capture light energy. However, it is unclear whether the drop in chlorophyll concentration is related to a decreased *de novo* production or increased degradation. Nevertheless, the results showed a decline in carotenoid content. The chlorophyll content of *V. radiata* and *E. coracana* was reduced by the aqueous extract of *A. muricata*. The presence of allelochemicals in *A. muricata* was responsible for the decreased chlorophyll concentration observed in this study. Frabboni *et al.* [36] and Ojija *et al.* [37] have reported similar findings. Chlorophyll photooxidation takes place due to a lack of carotenoid [38,39]. Reduced seed germination in *V. radiata* and *E. coracana* might be caused by the allelopathic stress of different extract concentrations resulting from different abnormalities in metabolic activities and cell division abnormalities due to the effect of *A. muricata* (Figs. 5 and 6).

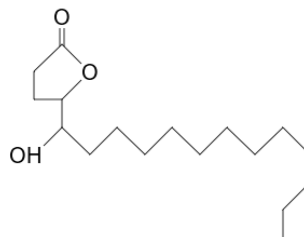
The biochemicals such as protein, amino acid, carbohydrate, peroxidase, and catalase of *V. radiata* and *E. coracana* seedlings were studied. In *V. radiata* (Table 2), amino acid (89.90%) and carbohydrate (71.09%) were reduced highly, followed by catalase (65.83%), protein (68.96%), and peroxidase (54.54%) at 50% concentration treatment of *A. muricata*, whereas lower concentration (1%) extract treatment stimulation of biochemicals as noticed. The allelochemicals of *A. muricata*, such as lignoceric acid, stearic acid, genistein, glycitein, homoorientin, isoferulic acid, kaempferol, quercetin 3-O-glucoside, quercetin 3-oneohesperidoside, quercetin 3-O-robinoside, quercetin-

Table 1: Structure of some of the allelochemicals produced by *A. muricata*.

S. no.	Chemical compound	Structure
1.	Isoferulic acid	
2.	Kaempferol	
3.	Genistein	
4.	Hydroxycinnamic acid	
5.	Gentisic acid	
6.	Quercetin3-O-robinoside	

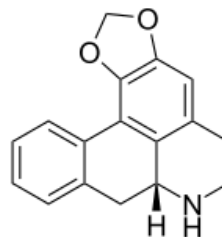
7.

Muricatacin



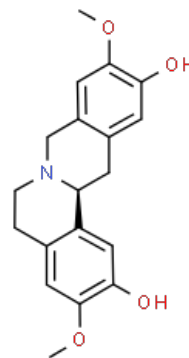
8.

Anonaine



9

Coreximine



10.

Taxifolin

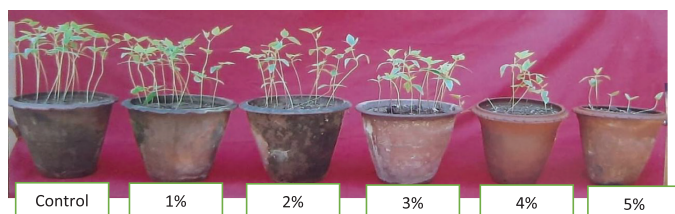
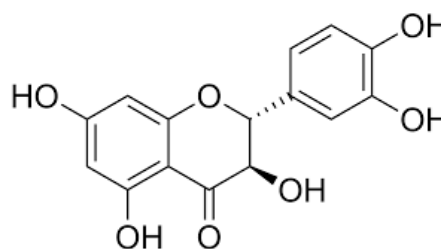


Figure 5: Allelopathic effect of *A. muricata* on seedling growth of *V. radiata*.

O-rutinoside, quercetin 3-O-a-rhamnosyl, robinetin, etc., inhibited the synthesis of biochemicals of test seedlings. In *E. coracana* (Table 3) seedlings, at all extract treatments only inhibition was noted. For the lower concentration (1%) extract treatment, very less amount of inhibition was observed. Protein (0.96%), amino acid (4.59%),

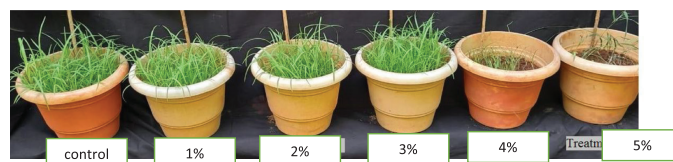


Figure 6: Allelopathic effect of *A. muricata* on seedling growth of *E. coracana*.

carbohydrate (2.51%), peroxidase (0.74%), and catalase (1.85%) were recorded over control. The inhibition of the test seedlings was different in the same extract treatment. From this experiment, it was observed that the inhibition or stimulation of seedling growth, germination, and biochemicals were different in different species by the influence of allelochemicals of *A. muricata*. Allelochemicals affect the production,

Table 2: Allelopathic potential of *A. muricata* on protein, amino acid, carbohydrate, peroxidase, and catalase (mg/g fr. wt.) constituents of *V. radiata*.

Extract concentration	Protein (mg/g fr. wt.)	Amino acid (mg/g fr. wt.)	Carbohydrate (mg/g fr. wt.)	Peroxidase (mg/g fr. wt.)	Catalase (mg/g fr. wt.)
Control	0.145	0.099	0.128	0.198	0.12
1%	0.149 (2.76)	0.109 (10.10)	0.13 (1.56)	0.203 (2.52)	0.124 (3.33)
2%	0.132 (-8.96)	0.084 (-15.15)	0.11 (-14.06)	0.189 (-4.54)	0.111 (-7.5)
3%	0.12 (-17.24)	0.064 (-35.35)	0.087 (-32.03)	0.162 (-18.18)	0.095 (-20.83)
4%	0.097 (-33.10)	0.047 (-52.52)	0.061 (-52.34)	0.143 (-27.78)	0.069 (-42.5)
5%	0.045 (-68.96)	0.01 (-89.90)	0.037 (-71.09)	0.09 (-54.54)	0.041 (-65.83)

Data in parenthesis indicates % increase/decrease over control.

Table 3: Allelopathic potential of *A. muricata* on protein, amino acid, carbohydrate, peroxidase, and catalase (mg/g fr. wt.) constituents of *E. coracana*.

Extract concentration	Protein (mg/g fr. wt.)	Amino acid (mg/g fr. wt.)	Carbohydrate (mg/g fr. wt.)	Peroxidase (mg/g fr. wt.)	Catalase (mg/g fr. wt.)
Control	0.209	0.087	0.159	0.134	0.108
1%	0.207 (-0.96)	0.083 (-4.59)	0.155 (-2.51)	0.133 (-0.74)	0.106 (-1.85)
2%	0.19 (-9.09)	0.07 (-19.54)	0.142 (-10.69)	0.12 (-10.45)	0.083 (-23.15)
3%	0.167 (-20.09)	0.05 (-42.52)	0.126 (-20.75)	0.105 (-21.64)	0.062 (-42.59)
4%	0.138 (-33.97)	0.03 (-65.51)	0.095 (-40.25)	0.087 (-35.07)	0.043 (-60.18)
5%	0.093 (-55.50)	0.009 (-89.65)	0.036 (-77.35)	0.056 (-58.21)	0.011 (-89.81)

Data in parenthesis indicates % increase/decrease over control.

functions, contents, and activities of various enzymes in diverse ways. Peroxidase and catalase can be suppressed by taxifolin, vitexin, caffeic acid, hydroxycinnamic acid, gentisic acid, hydroquinone, gallic acid, tannin, flavonoids, and phenolic compounds, which can also reduce the synthesis of amylase and acid-phosphatase in the endosperm. Sofi *et al.* [40] have thoroughly documented the link between root length and plant failure to compete and search for water and minerals from the soil. Their works support the present findings.

All phenolic acids can impact the integrity of DNA and RNA [41,42]. Many phenols and alkaloids, as well as ferulic acid and cinnamic acid, can limit protein synthesis. In barnyard grass co-cultured with allelopathic rice or treated with rice-produced phenolic acids, the expression levels of miRNAs related to plant hormone signal transduction, p53 signaling pathways, nucleotide excision repair, and the peroxisome proliferator-activated receptor were increased [43]. According to Kato-Noguchi *et al.* [44], the rice allelochemicals momilactone A and B may prevent *Arabidopsis* seeds from germinating by preventing the breakdown of the storage proteins and cruciferina. These studies support the present findings. Allelochemicals released by donor plants affect receiver plants, and receiver plants respond to the donor plants by changing gene expression. Most phenolic allelochemicals can stimulate Indole-3-Acetic Acid (IAA) oxidase activity and inhibit the reaction of peroxidase with IAA, bound gibberellic acid or IAA to influence endogenous hormone levels [45]. Other plant species' growth and development are inhibited by extracts from all parts of the plant.

4. CONCLUSION

The present investigation showed that aqueous leaf extract of *A. muricata* at different concentration levels inhibited seedling growth

of *V. radiata* and *E. coracana*. However, a low concentration (1%) stimulated germination, seedling length, pigments, starch, protein, amino acid, carbohydrate, peroxidase, and catalase contents of the *V. radiata* seedlings only. Therefore, the study indicated the beneficial and harmful effects of *A. muricata*. This plant contains phytochemicals, primarily acetogenins, alkaloids, and phenols. These allelochemicals highly affect the crops. *Annona muricata* allelochemicals play an important role in the investigation of appropriate farming systems, as well as in the control of weeds, diseases and insects, the alleviation of continuous cropping obstacles, and allelopathic cultivar breeding. Allelochemicals can also be used as ecologically friendly herbicides, fungicides, insecticides, and plant growth regulators, making them extremely useful in sustainable agriculture. Between the two test crops, more inhibition was observed in *E. coracana* than *V. radiata* seedlings at 5% aqueous leaf extract treatment. This experiment concluded that the inhibition or stimulation of the seedlings was concentration-dependent and varied in different species. Detailed field studies need to be undertaken to ascertain whether the inhibitory effects noted in the pot culture experiments are expressed in the field too.

5. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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