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Effect of Isolated Bioactive Compounds from Erythroselinum atropurpureum against Biofilm Forming Pathogens

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Abstract

Biofilm is the community of microbial cells that attach to living or non-living things. Biofilm members communicate, exchange experiences, and virulence factors with each other, which called quorum sensing. The main objective of this study was to evaluate the effect of isolated bioactive compounds from Erythroselinum atropurpureum against pathogens microbes. The bark of Erythroselinum atropurpureum was extracted using hexane, ethyl acetate, and methanol for 72h soaked at room temperature. Antibiofilm was tested by test tube, swarming motility, and inhibition of violet colour production test methods. The results indicate that ethyl acetate extract of Erythroselinum atropurpureum and its purified compounds exhibited the strongest antimicrobials, antibiofilm activity. The antimicrobials activity of the ethyl acetate extract against S. aureus and C. albicans ranged between 5.5-22.7mm inhibition zones. The test tube methods indicate that 2.5mg/mL of extract were inhibited the biofilm formation of S. aureus. Anti-swarming activities were shown by 2.5mg/ml of the extract on P. aeruginosa, S. aureus and the highest inhibition of violacein production of S. aureus, P. aeruginosa, and C. albicans 78.6%, 69.7%, and 67.3% respectively. The Erythroselinum atropurpureum examined in this study has been varying degrees of antimicrobial activity against bacterial and biofilm forms with some having good activity against both forms. This selected medicinal plant might be potential candidates for further investigation to isolate, synthesis antimicrobial compounds and determine the mechanism of activity.

Keywords: Antimicrobials, Bioactive**,** Biofilm, *Erythroselinum atropurpureum* Pathogen

1. INTRODUCTION

The search for new antimicrobial agents is providing effective control a large number of infectious diseases (Ling *et al*., 2015). As a consequence of the increment of multi-drug resistance bacteria because of microbial resistance, this day antibiotic treatments became more challenging to produce and clinically implement. The documented antibiotic resistance in recent research is required for the development of different target processes in the pathogen. Such target systems are quorum sensing and biofilm formation (Tanver *et al*., 2017). Quorum sensing consists of cellto-cell communication that depends on the production and response to a small diffusible molecule called auto-inducers. This can lead to the formation of biofilm and causing other virulence factors which are problems for food safety and infection diseases (Tanver *et al*., 2017).

Considering the impact of quorum sensing and biofilm, several strategies (cleaning agent, phages, irradiation, essentials oil, etc.) have been developed in food factory and drug discovery (Tanver *et al*., 2017). To prevent spoilage of food and bacterial infection by using medicinal plants are safe and non-toxic regarding health status. Further, blocking of signal transduction can be achieved by using an antagonist molecule capable of competing or interfering with the native AcylHomoserine Lactone (AHL) signal for binding to the LuxR (autoinducer-responsive transcriptional activator) type receptor (Koh *et al*., 2013). Competitive inhibitors are structurally like the native AHL signal and can bind to and occupy the AHL-binding site but will fail to activate the LuxR-type receptor. The non-competitive inhibitors show little or no structural similarity to AHL signals and these molecules bind to different sites on the receptor protein (Koh *et al*., 2013). Thus, quorum sensing inhibitors that can either the target synthesis of the cell signaling molecules or block these signaling systems can be used to prevent spoilage of food and biofilm formation by food-related bacteria (Tanver *et al*., 2017).

The use of natural products as a therapy against infectious diseases is a traditional therapeutic measure especially in developing countries as they contain a combination of the potential antimicrobial compounds instead of a single purified molecule (Packiavathy *et al*., 2012). Thus, it has become inevitable to search for new and safer anti-QS compounds from natural products. Quorum sensing inhibitors have been reported in various natural products and medicinal plant species, in fruits and spices, and phytochemicals (Packiavathy *et al*., 2012).

In Ethiopia, local people use different medicinal plants to preserve food, treat infectious diseases, and use them as a source of food traditionally. These plants were collected from wild habitats and prepared either in dry form or fresh form remedies. Most remedy preparations did not have additive substances while the remaining have different additive substances like honey, sugar, butters, and oil for the treatment of a single ailment (Mersha Ashagre, 2011).

Medicinal plants are popular for their new bioactive compound and therapeutic values. The antimicrobial activities of some of the tested crude extract and their constituents have been development of a novel drug based on the antimicrobial activity of plant crude extracts. However, there are very few studies focusing on the effect of bioactive compounds from plant extracts on biofilm-forming pathogens. Based on the traditional value of medicinal plants, this study was designed to evaluate the effect of isolated bioactive compounds from the locally available medicinal plant of *Erythroselinum atropurpureum* against biofilm-forming pathogens, and antimicrobial properties.

2. Materials and Method

2.1. Study Area

The samples for this study were collected from Bule Hora District, West Guji Zone, Oromia regional state, which is located is 467 km away from Addis Ababa to the South and 100Km North of Yabello, the capital city of Borana Zone. This District lies between latitudes 50 26'and 50 52'North and longitudes 370 56'and 380 31'East with a total area of about 488,861.3 hectares or 48,886.1 km² of which 77.1% is middle land and 22.9% is lowland and the altitudinal range lies between 1465-2300 ma. S.l.

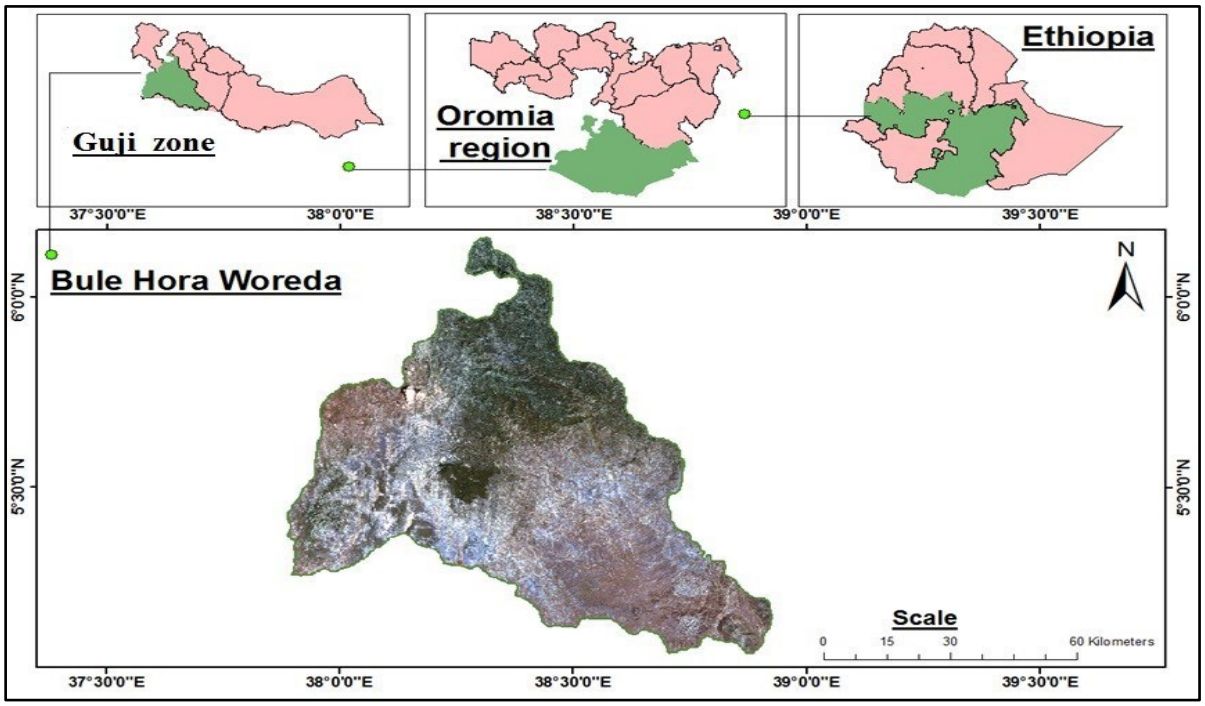

Figure 2.1. Map of the study area

2.2. Plant Specimen Collection

Erythroselinum atropurpureum (locally named as Baattii) (2Kg) were collected from Bule Hora district, West Guji Zone. The fresh sample was packed in plastic bags and transported into the biology laboratory.

Figure 2.2. Photo of plant sample collected for the study.

2. 3. Sampling Techniques and Research Design

The study was targeted around Bule Hora district, West Guji Zone, Oromia regional state. The sampling technique was determined using random sampling techniques. Random sampling techniques were employed to select the sites from where the plant materials were collected. The study would be conducted using Experimental works of the following schematic diagram.

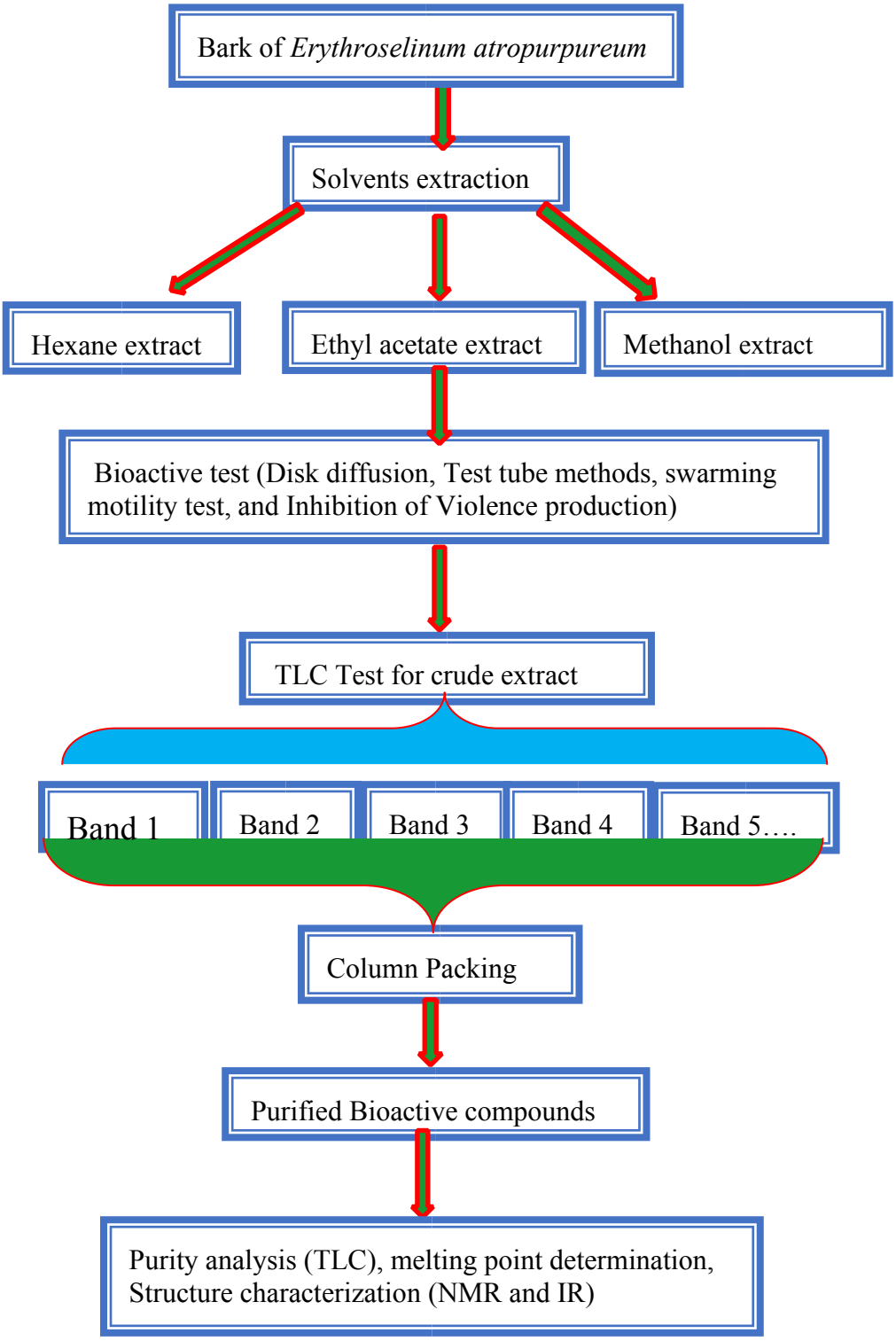

Figure 2.3. Research design based on the experimental procedures.

2.4. Specimen Preparation and Extraction

The sequential extraction method described by Ncube *et al.*, (2008) was applied with slight modifications as follows. The plant samples were washed thoroughly under running tap water to remove dirt, dust, and insects and dried in dry air under a shade. The chopped and crushed bark of *Erythroselinum atropurpureum (*262.7g) with fine size was soaked in 1.5 L of hexane for 72 hours with occasional swirling to ensure thorough sequential extraction. The soaked crude powder was filtered, concentrated using rotary evaporator, and dried for two days in a bottled in laminar flow hood, and weighed. Next, the left crude was also soaked in 1.5 L of ethyl acetate for 72 hours with occasional swirling to ensure further extraction. The extract was filtered and concentrated using rotatory evaporator, dried, and weighed. Finally, it soaked with ethyl acetate in 1.5 L methanol for 72 hours with occasional swirling and filtered, concentrated, and weighed in similar ways. The extracts were stored in the freezer at 4°C until tested for antimicrobials and antibiofilm activities test.

2.5. Reference Pathogens

Two reference strains for human pathogenic bacteria representing Gram-positive (*S. aureus* ATCC 6538) and Gram-negative (*E.coli* ATCC 9027) bacteria and a pathogenic fungus (*Candida albicans* ATTCC 64550) were obtained from the Ethiopian Institute of Biodiversity and used for the antimicrobials and antibiofilm activity test.

2.6. Antimicrobial Susceptibility Test

The antimicrobial activity test of the crude extracts was against Gram-positive (*S. aureus)* and Gram-negative bacteria (*P. aeruginosa) and Fungus (C. albicans)* were carried out by disk diffusion method. The Muller Hinton Agar and Sabroud Dextrose Agar were prepared for each organism according to the manufacturer's instructions. Briefly, the recommended measurements of solid Muller Hinton agar and Sabroud Dextrose Agar powder were mixed in distilled water aseptically; the solution is then boiled on an electric stove with stirrer in it for mixing. Then 20ml of sterile Muller Hinton Agar (maintained at 45-50^{o}C in a molten state), and Sabroud Dextros Agar solution were poured into sterilized Petri dishes separately and set aside. After solidifying, 0.5McFarland standard of test organisms were spread on Muller Hinton Agar plate and *Candida albicans* were spread on Sabroud Dextrose Agar. Then

previously prepared filter paper discs were placed on the surface of the agar plate at equal distance from each other and at a 15 mm distance from the edge of plate. The discs were placed with a dispensing apparatus and each disc was pressed down to ensure complete contact with the agar surface and, distributed evenly. Each plate was inverted and placed in an incubator set at 37^{0}c for 24 hours after the discs are applied for bacteria. The antimicrobials susceptibility was evaluated by measuring the zone of complete inhibition using a plastic ruler in mm.

2.7. Antibiofilm Activity Test

2.7.1. Test Tube Method

Reference pathogen (0.1mL) was obtained by adjusting turbidity to 0.5McFarland standards was transferred to test tubes containing 10mL Luria Bertain (LB). 200■ of crude extract of bark of *Erythroselinum atropurpureum* were added separately to the test tubes containing the test organisms and incubated at 37°C for 72 hours. The medium was removed and the tubes were washed with distilled water, air dried, and biofilm formation in the tubes was tested using crystal violet. All tests were carried out in triplicates and results of the test were recorded.

2.7.2. Swarming Motility Test

Swarm plates were prepared using nutrient agar containing 0.5% peptone, 0.2% yeast extract, and 1.0% glucose per 100ml of distilled water. Two hundred and fifty microliter (250µl) of plant extracts were added to 10ml semisolid agar, gently mixed, and poured immediately onto the surface of a 10ml of pre-warmed agar plate as an overlay. Twenty microliters of standardized inoculums were placed on the center of the plate. A similar volume of test organism was placed on the center as negative control plate (overlay agar without extract) and the plates were incubated overnight at 37^{o}C upright position for 30hrs.

2.7.3. Flask Incubation Test for Quantification of Violence in Production

Two hundred microliters of test organism were inoculated in Erlenmeyer flasks containing 20ml of LB supplemented with extract of *Erythroselinum atropurpureum* 2.5mg/ml, 5mg/ml, and 10mg/ml concentrations. The flasks were incubated at 37°C in a shaking incubator for 30hr. Two milliliters of each culture were transferred to a test tube and centrifuged at 1500 rpm for

15min to precipitate the insoluble pigment. The pellet was re-suspended in 2ml of dimethyl sulfoxide

(DMSO) and homogenized by vortexing. Violacein absorbance at 585nm was determined using UV–visible spectrophotometer. The negative control sample consisted of incubating the test organism in LB broth without adding extract and violacein absorbance was determined with the same procedure of test samples. Inhibition was calculated with respect to control and triplicate measurements were performed, and the value was determined by the following formula:

$$\text{Inhibition of violacein production} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \times 100\ \%$$

2.8. Data Analysis

The data obtained in this study were subjected to analysis of variance (ANOVA). Excel and Minitab version 17 statistical software were used and comparisons of mean and correlation were performed. The value of $p < 0.05$ is used as statistically significant.

3. RESULTS AND DISCUSSION

3.1. Yield of Extracts

The highest percentage yields were extracted from *Erythroselinum atropurpureum* by ethyl acetate which was 1.72% followed by methanol extract of 1.32% and by hexane extract of 1.17% respectively.

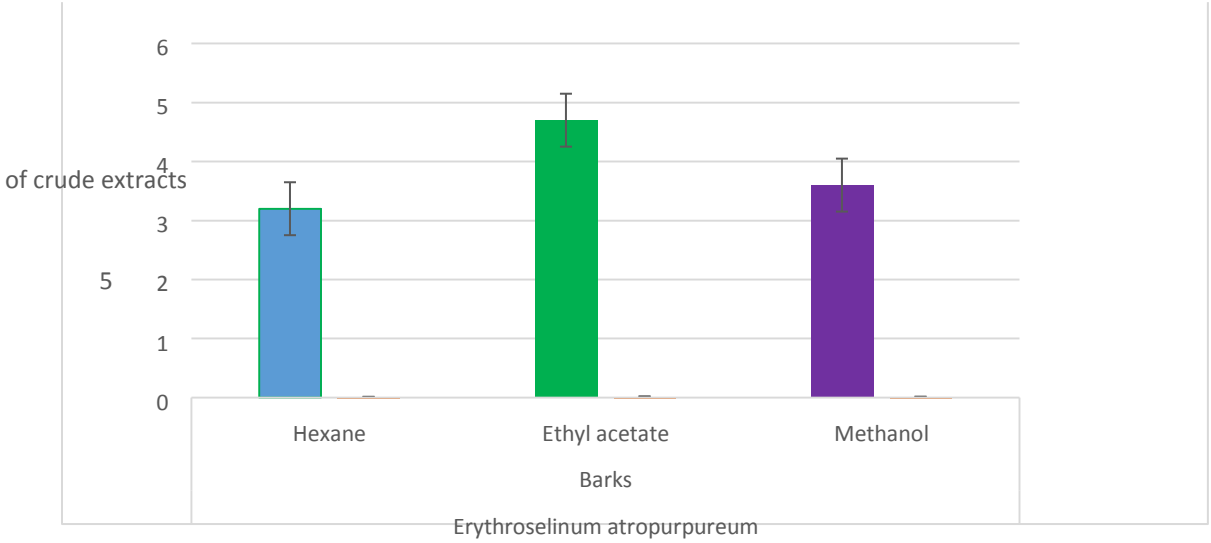

Figure 3.1. Percentage of yields of extracts were obtained from the Bark of *Erythroselinum atropurpureum*

3.2. Antimicrobial activity test

The Ethyl acetate and Methanol extracts of *Erythroselinum atropurpureum* showed antibacterial activity on *S. aureus* at 5 mg/mL and antifungal activity on *C. albicans* at 2.5 mg/mL concentrations (Figure.3.2). The results revealed that *Erythroselinum atropurpureum* crude extract inhibit the growth of *S. aureus, C. albicans* but not inhibit growth of *P. aeruginosa.*

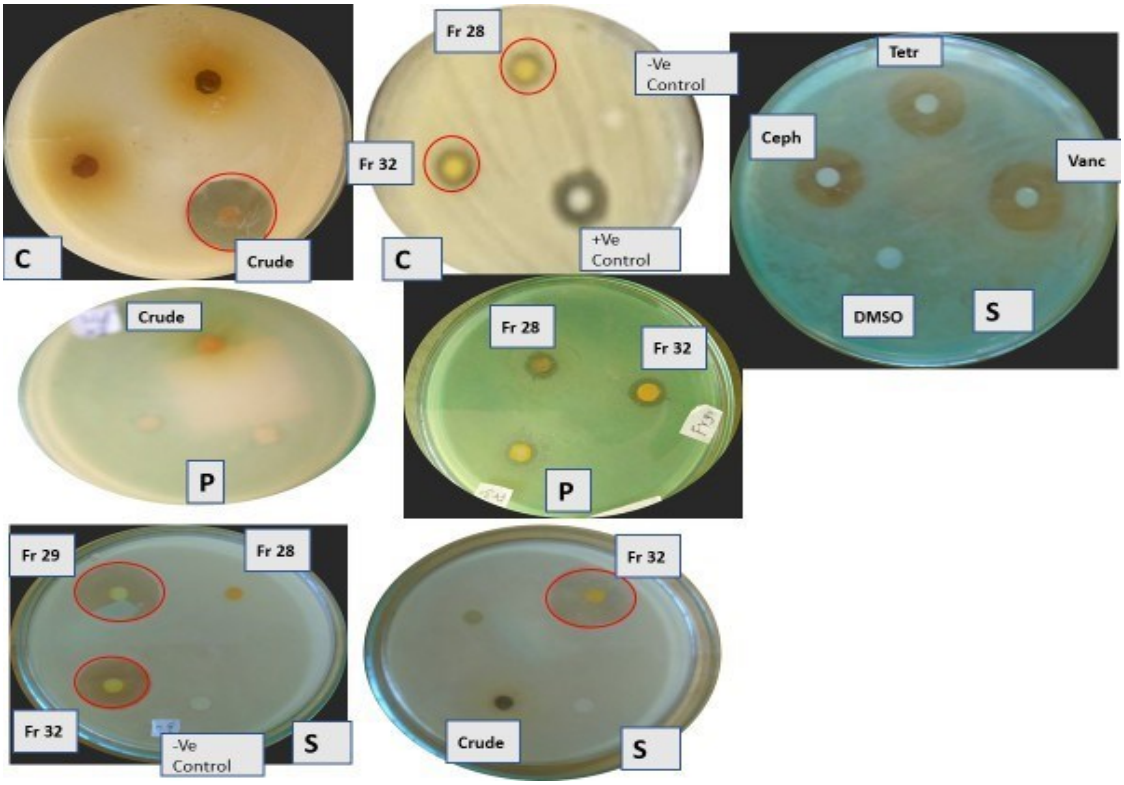

Figure 3.2. Antimicrobial activities of bark crude extract of *Erythroselinum atropurpureum*, against *C. albicans, S. aureus* at 5mg/ml and 2.5mg/ml concertation, (C) *C. albicans*, (P) *P. aeruginosa* (S) *S.*

aureus, (Fr) fractionated compounds, and circle shows the diameter of inhibition zone.

Table 3.1. Effect of crude extract of *Erythroselinum atropurpureum* at 2.5mg/ml against *S. aureus, P.*

aeruginosa, C. albicans.

Plant species		**Zone of inhibition in mm**

	Extraction Chemical	*S. aureus*	*P. aeruginosa*	*C. albicans*
Erythroselinum atropurpureum	Ethyl acetate	16±1.5	NI	22±1.98
	N-hexane	NI	NI	6.5±0.34
	Methanol	6±1.22	NI	14±0.67

The Methanol extract of *Erythroselinum atropurpureum* was showed the highest degrees of antibacterial and antifungals activity ranged between 5.5-22.7mm inhibition zones. The largest zone of inhibition was observed for *Erythroselinum atropurpureum* (ethyl acetate extract) with an inhibition zone of 20mm for *C. albicans,* 16mm for *S. aureus*. *Styphalococcus. aureus and C. albicans* were found to be susceptible to *Erythroselinum atropurpureum* extracted by ethyl acetate as the zone of inhibition diameters (16mm and 20mm for *S. aureus, and C. albicans*. *Pseudomonas aeruginosa* was resistant to all extract, but purified compound by column inhibit the growth of *P. aeruginosa* and all our test organisms. The hexane extract did not show any antibacterial activity towards both bacteria and fungus.

The bioactivity results were considered evidence of the presence of more compounds in the crude extract of ethyl acetate, compared to the methanol and hexane extracts of *Erythroselinum atropurpureum*, the probable reason is that the compounds in bark of *Erythroselinum atropurpureum* were more soluble in ethyl acetate as compared to methano l and hexane. However, different activities against *S. aureus, P. aeruginosa, and C. albicans* were observed by fractionated compounds. The inhibition activity at different concentration showed different zone of inhibition by purified compounds from methanol extract of *Erythroselinum atropurpureum*. This purified compound from methanol extract of *Erythroselinum atropurpureum* used in this study showed variable antibacterial activities against *P. aeruginosa* at concentrations ranging from 10 to 2.5mg/ml.

3.3. Anti-Biofilm Activity Test

3.3.1. Test Tube Method

Strong biofilm formation by *C. albicans* and *P. aeruginosa* compared with control as observed by the heavy crystal violate pigment remaining on the test tube. *Staphylococcus aureus* showed little biofilm formed when they were treated with methanol extract of *Erythroselinum atropurpureum*.

Figure 3.2 is showing the adherence of biofilm stained by crystal violate. The initial cell attachment was significantly inhibited in the case of *Erythroselinum atropurpureum* against *S. aureus* at 2.5mg/ml concentration.

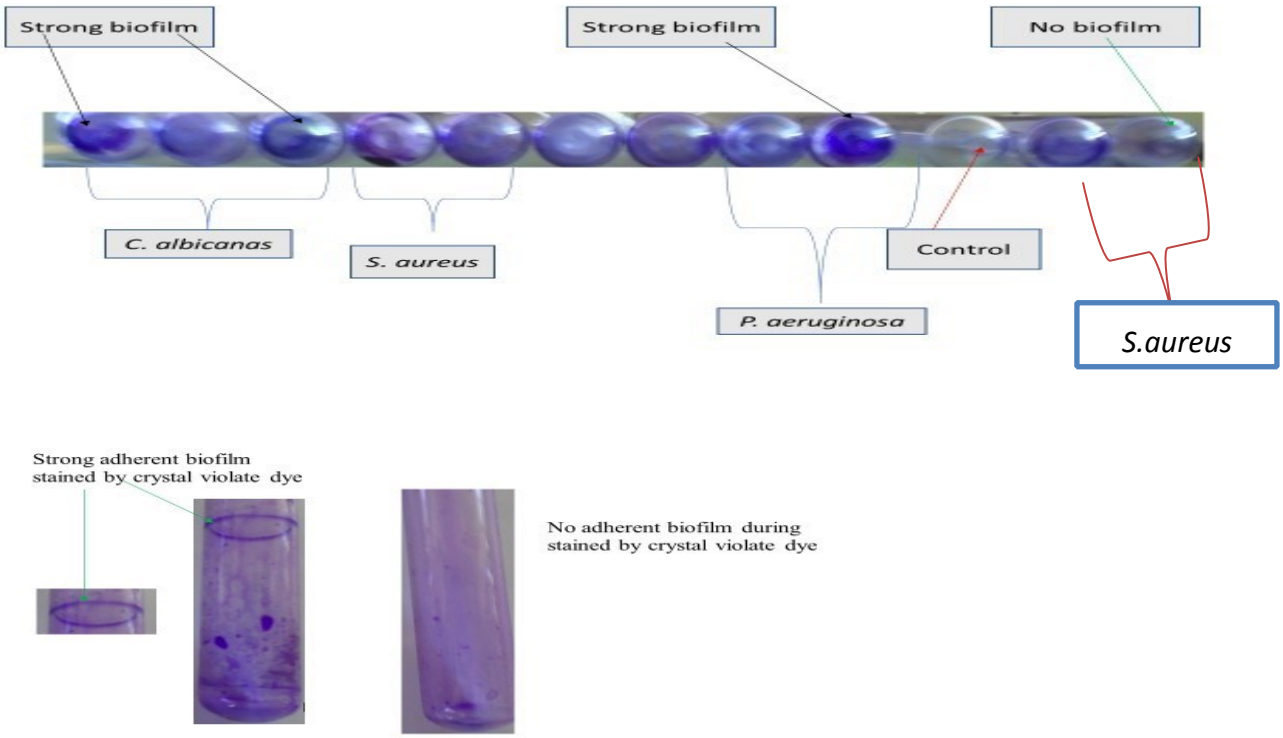

Figure 3.3. Anti-biofilm activities of *Erythroselinum atropurpureum* against *C. albicans*, *P. aeruginosa*, *and S. aureus* crystal violate staining method.

The crystal violet assay indicated that the effect of *Erythroselinum atropurpureum* extract on initial cell attachment. To ensure a concentration that is affecting the microbial attachment, concentrations at 10mg/ml, 5mg/ml, and 2.5mg/ml for anti-biofilm assay were used. Crystal violates staining method is easily and widely used to measure both the formation and inhibition of biofilms. The methanol extract of *Erythroselinum atropurpureum* at a concentration of 10mg/ml is the lowest concentration, which inhibited on *S. aureus* biofilm formation.

3.3.2. Swarming Motility Inhibition

In the swarming motility assay, overnight cultures of the test bacteria were point inoculated at the centre of the medium consisting of 1% tryptone, 0.5% NaCl, and 0.3% agar with 10mg/ml concentrations of extracts. The plates were incubated at 30 °C in upright position for 24h. Following swarm zones of the bacterial cells swarming migration was observed. Figure 3.4 indicates that methanol extract of *Erythroselinum atropurpureum* has a significant influence on the swarming motility but concentration different activity among different microorganisms. Best antiswarming activities were shown by 10mg/ml of the extract on *P. aeruginosa*, *S. aureus.*

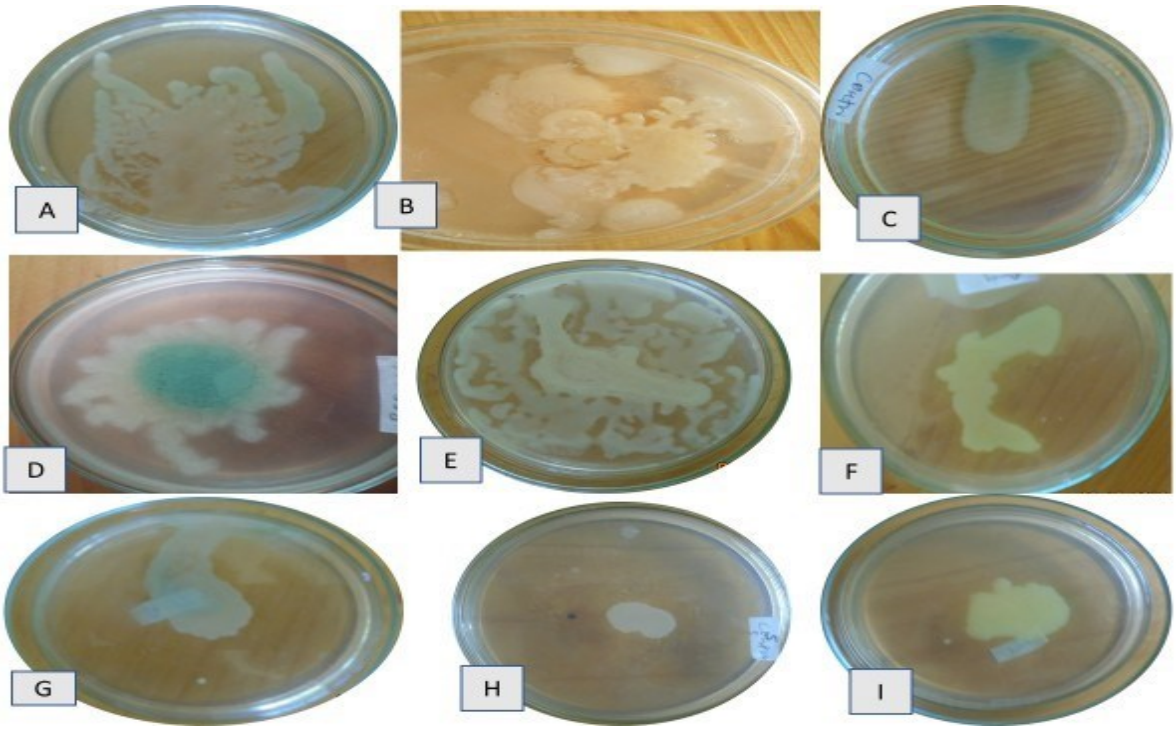

Figure 3.4. The migration distance of *S. aureus*, *P. aeruginosa C. albicans* were observed while they were incubated for 24 hours at 2.5mg/ml concentrations of *Erythroselinum atropurpureum* extract and control: without extract. (A and E) *P. aeruginosa,* (B) *S. aureus,* (C, D and G) *C. albicans*, (F and I) control *P. aeruginosa (*H) control *S. aureus.*

3.4. Flask Incubation for Violacein Inhibition Production

Inhibition of purple pigment by *S. aureus,* and *C. albicans* on the presence of the extract of *Erythroselinum atropurpureum* are an indication of quorum sensing inhibiting. The inhibitory effect of the *Erythroselinum atropurpureum* extract on violacein pigment production was

measured and quantified by spectrophotometers (Table 3.2 absorbance measurements). A concentration of 10mg/ml extract inhibited about 72.6 % violacein production which has about 4 times higher inhibition than 5mg/ml concentrations inhibition (16.2% inhibition). This value was calculated by the formula written under 2.7.3 methods.

Table 3.2. Inhibition of violacein production by *Erythroselinum atropurpureum* extract

	Plant species	Reference pathogens	**Concentration mg/ml**		% of inhibition violacein production at 10mg/ml
			5	**10**	
			Absorbance at 585nm		
1	*Erythroselinum atropurpureum*	*P. aeruginosa*	0.55	0.20	69.74%
		S. aureus	0.52	0.17	78.6%
		C. albicans	0.57	0.19	67.35%

NB: the absorbance of control was 0.62, since the control has Luria Bertain and 20 microliters of test organisms, but the test sample contains Luria Bertain + 20 microliters of test organism +5 and 5mg/ml of crude extract respectively.

4. DISCUSSION

The increase of multidrug resistance has been creating several problems. To solve such problems there are new antibiotics were being extracted either from medicinal plants or from antibiotic producing microorganisms. One of the most common approaches is the search for the easiest and the safest antibiotics for human. Medicinal plants are known to be the safest and their extracts do not pose selective pressure issues and unlikely to cause resistance problems.

One reason for choosing plants as a source of new antibiotics is medicinal plants are locally available. In the entire world flora, 250,000 species have been identified and used for curative purposes (Patwardhan *et al.*, 2005). This number represents only 15% of those species that have been effectively investigated and found useful (Okeke *et al.*, 2005). Consequently, there is a staggering over 85% of higher plants, which are yet to be investigated.

Crude extracts of the plants may have mixtures of active compounds whose overall bioactivity is usually greater than individual compounds (Tanver *et al.*, 2017). The percentage of extraction yields either increase or decrease with the ratio of solvent, the temperature of extraction and extraction methods (green extractions, microwave-assisted solvent extraction, ultrasound, etc) (Tanver *et al.*, 2017).

Bioactivity of plants extract significantly was varied based on the solvents used for extraction and depends on the geographical source (Cervenka *et al.*, 2006), harvest time, storage conditions, soil conditions, drying method, and route extract (Bernard *et al.*, 2014). The benefit of plant properties against microorganisms can only be achieved by using a specific solvent and solvent concentration in extracting the plant materials.

Hence, in this study, ethyl acetate was selected as the extraction solvent because of the crude extract by ethyl acetate showed highest bioactivity against gram-positive bacteria (*S. aureus* and *C. albicans* and highest percentage of yield. Further, ethyl acetate extraction is widely used to obtain crude extracts of phytochemicals from plant. The concentration of the solvent could affect the antimicrobial activity, it is necessary to select the appropriate solvent and its concentration.

Several studies are conducted to investigate the biological activities of plants (Bernard *et al.*, 2014). Most of these studies tried to determine the antimicrobials activates of extracts obtained from plant materials. However, this study focused to evaluate the effect of isolated bioactive from *Erythroselinum atropurpureum* against biofilm forming microbes of extracts and its purified compounds to explore the possibility of finding out new methods for prevention or treatment of infectiou s disease caused by biofilm forming microorganisms.

4. In Vitro Effect of Plant Crude Extracts on Biofilm Formation

The present study focused on the anti-biofilms and quorum sensing (QS) inhibitory effect of isolated bioactive compounds from the bark of *Erythroselinum atropurpureum*. The activity of the extracts on inhibition of biofilm formation was tested using the crystal violet method, which is widely used by microbiologists. It is inexpensive and can be repeated many times to ensure accurate results (reproducible). The extracts used in this study showed antibacterial activity against *S. aureus* and *P. aeruginosa* at 10 and 5mg/ml concentration. The reason for the selection of *S. aureus* and *P. aeruginosa* in this assay is the fact that the biofilms forming ability of this

bacterium contribute to its colonization in causing acute infections as well as its presence in burned tissue surrounding blood vessels and adipose cells (Schaber *et al*., 2007). Various results were obtained on inhibition of biofilm for *Erythroselinum atropurpureum*. *Erythroselinum atropurpureum* showed prominent antimicrobial and anti-biofilm activities. The result obtained by Latakian *et al*., (2016), agrees with this study. In this study, the *Erythroselinum atropurpureum* extracts test prevented the formation of biofilm at concentration of 10mg/mL, like a study by Pratiwi (2015), which prevented the formation of biofilm at concentration of 9mg/ml.

A low concentration of the extract may be required to prevent biofilm first attachment, while higher concentration to disrupt preformed biofilm (Stewart, 2002). This study indicated that most plant extracts have the antibacterial coupled with anti-biofilm activity, therefore, may prove helpful for developing biofilm inhibitors and increase the effectiveness of infectious diseases treatment. In this study, *Erythroselinum atropurpureum* showed remarkable potential as anti-biofilm agent, as it was active upon *P. aeruginosa, S. aureus, C. albicans* in the planktonic state, no articles on the anti-biofilm activity of extract of *Erythroselinum atropurpureum* was published, however, it failed to reduce the quorum sensing (QS) in most assays.

The first discovered natural products the inhibited quorum sensing (QS) and biofilm maturation in gram-negative bacteria are the halogenated furanones from *Delisea pulchra*, and another quorum sensing (QS) compounds such as cyclic sulphur derivatives obtained from garlic and patulin produced by *Penicillium* species (Persson *et al*., 2005). *Erythroselinum atropurpureum* extract showed strong anti-biofilm activity against *S. aureus* and *P. aeruginosa* compared to the untreated control. These medicinal plants could be used to manage *Pseudomonas* pathogenesis and hinder its dissemination. To the best of our knowledge, no reports are available regarding the anti-biofilm activity of *S. aureus P. aeruginosa* and *C. albicans* by *Erythroselinum atropurpureum* extract.

The anti-QS potential of plant extracts to explore the potential to possible use in controlling detrimental pathogenic bacteria such as *S. aureus P. aeruginosa*. This results in different systems/pathways of quorum sensing by *S. aureus P. aeruginosa* and *C. albicans*. Therefore, there are different mechanisms of quorum sensing inhibition by active component from plant extract, either by inhibition the production of the virulence factor violacein, inhibition of two QS system effective compounds, and inhibition of QS system receptors (Loughlin *et al*., 2013).

We investigated that the extracts inhibited *S. aureus*, *P. aeruginosa* and *C. albicans* motility as a means for determining the inhibition of quorum sensing as an alternative strategy for controlling bacterial virulence as suggested by Vasavi *et al.*, (2014). The swarming motility is important for cystic fibrosis acute infections, a common cause of severe nosocomial infections, and involved at early stages of biofilm formation. Three major forms of motility are displayed by *S. aureus* and *P. aeruginosa* (swarming growth on semisolid, swarming growth on solid surfaces, and flagellummediated swimming in aqueous environments). The swarming assay depends on factors such as nutrient composition, agar type and composition, sterilization protocol (e.g., autoclaving), and semi-solid media curing among others, thus, it is highly sensitive to environmental factors. The edge of the colonies in smarming motility is highly irregular compared with control. Flagellarmediated swimming motility is associated with biofilm formation by instigating the cell-to-surface attachment and plays an important role in the virulence of pathogens (Wu *et al.*, 2014).

Erythroselinum atropurpureum extract reduced the swarming motility of *S. aureus* and *P. aeruginosa.* It might be because of the phytochemicals of *Erythroselinum atropurpureum* on flagella-related processes, namely, flagella biosynthesis, rotation, rafting, and chemotaxis, etc. Interestingly Tremblay and Deziel (2008) reported that swarming motility assays of *P. aeruginosa* are influenced by incubation temperature, % agar, pH, and drying time under laminar flow. In this study, we used molten soft top agar for all experiments about swarming motility. Now it is well established that most of these compounds have antimicrobial and even antifungal activity (Zhang *et al.*, 2014). Interestingly, studies associated with some natural products have shown that plant extracts with poor antimicrobial properties could also be effective against bacterial biofilms (Upadhyay, 2014).

5. Conclusion

The organic crude extract and isolated compounds from *Erythroselinum atropurpureum* were active against *C. albicans, P. aeruginosa and S. aureus.* The highest antimicrobial activities against *C. albicans* and *S. aureus* was observed for Ethyl acetate and methanol *extract of Erythroselinum atropurpureum*. The results obtained by ethyl acetate extracts of *Erythroselinum atropurpureum* have shown better inhibited biofilm formation by *S. aureus* and *P. aeruginosa due to* constitute of an interesting source of alkaloids, saponin, flavonoids, and tannin for anti-biofilm agents in the

development of new strategies to treat infections. Indeed, *Erythroselinum atropurpureum* extracts exhibited inhibition of swarming motility against *P. aeruginosa, S aureus,* and *C. albicans*. This result may stimulate further biological research on these and other under-explored plant species, which is native to the study area.

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Conflicts of Interest: The authors declare no conflicts of interest

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