Research Article

Antimicrobial Activity of Some Heterocyclic Compounds and Herbal Extracts on Plant Pathogens

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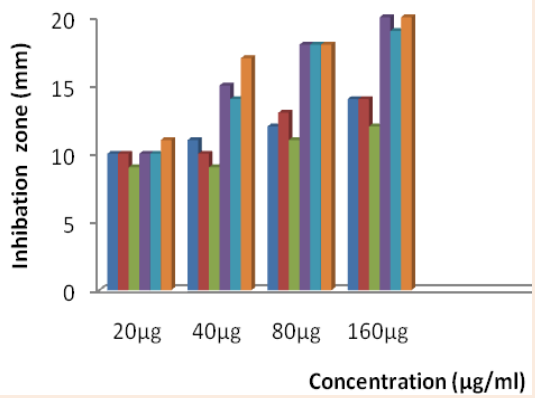
Abstract

The aim of this work was to evaluate the antimicrobial activity of synthesized heterocyclic compound (SHCs) like as (furan derivate(Fd) (1-furan-2-yl-thioxo-propenon) , thiophene derivate (Thd) (2-cyano-2-sc-thiophene-2-yl-[1,2]dithiol-3ylidene thioactamide)) and herbal extracts (HEs) like as (Cocklebur and Colocynth) against plant pathogens: three fungal strains (*Fusarium oxysporum* , *Rhizopus stolonifer* and *Aspergillius niger*), two bacteria strains (*Pseudomonas solanacearum* and *Erwinia cartovora*) and one actinomycetes strains (*Streptomyces scabies*) . The synthesized heterocyclic compounds were subjected to antimicrobial activity against various plant pathogenic microorganisms. Investigation of antimicrobial activity of the derivatives demonstrated the ability to inhibit Gram-negative microorganisms with zone of inhibition ranging from 10-14 mm, fungal inhibition zone ranging from 9-22 mm. All extracts (synthesizes and herbal) had strong antimicrobial activity against the evaluated pathogenic microorganisms.



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Introduction

In recent years increased attention to chemical compounds derivatives and medicinal herbs to use like as sources for the production of medical drugs and pharmaceuticals. Also has been used chemicals derived from furan and thiophene derived compounds are known compounds (Heterocyclic Compounds) have an impact on plant pathogen [1]. In general, SHCs are products exhibit miscellaneous biological activity, as well as antibacterial, antifungicidal, analgesic, anti-inflammatory activity [2-8]. These heterocyclic systems find wide use in medicine, agriculture and industry. More particularly and recently these types of compounds have been found in the treatment of plant pathogen and other chemotherapeutic diseases .Synthesized Heterocyclic compounds (SHCs) are organic compounds containing at least one atom of carbon, and at least one element other than carbon, such as sulfur, oxygen or nitrogen within a ring structure. These structures may comprise either simple aromatic rings or non-aromatic rings. Some examples are Furan and thiophene, many heterocyclic compounds, including some amines, are carcinogenic, these derivatives compounds have effectively a biological and pharmaceutical [9]. Many studies have addressed the effect of herbal extracts on microbial growth and thus the possibility of their use in the treatment of some pathogenic plant diseases [9,10] . Several studies were conducted on the effect of herbal extracts on Microbes [9]. These herbal was selected to provide in abundance in the local environment and their effectiveness in inhibiting the growth of microbes [11] .On the other hand was conducted studies on antimicrobial activity of heterocyclic compounds on plant pathogen, this study focus on the effect of extracts colocynth (bitter melon) and cocklebur on the growth of different types of plant pathogens (bacteria, fungi and stryptomyces).

Colocynth (Citrullus colocynthis Schrad) is medicinal plants that belong to Cucurbitaceae family , It is a powerful laxative and diuretic severe and called bitter apple to used to expel intestinal parasites in the treatment of jaundice and rheumatism, headaches, sciatica and Back pain and antiseptic properties used in the treatment of wounds, and the plant toxic if taken large quantities of the most important materials It is a very bitter glucosides, and resin materials and pectin Colocynthin effective it is colocynthen and Sapindales . Cocklebur (Xanthium strumarium) is medicinal plants that belong to Asteraceae Family, used in this work was cultivated in the experimental field of the faculty of agriculture farm, south valley university, Egypt. The leaves were separated, dried in a tray drier with air circulation at 45ºC, packed in dark plastic bags and stored in a domestic freezer at -5 ºC until extracted. This study aims to find out the antimicrobial activity of herbal extracts of plants (colocynth and cocklebur) and Heterocyclic compounds (sulfur compounds and oxygen compounds) on different types of plant pathogens.

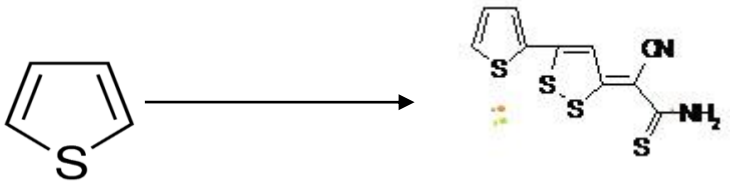
Experimental

Preparation of herbal extracts

The aqueous extract were prepared by adding 5 g of crushed leaves of cocklebur in 50 ml of distilled water and 5 g of milled colocynth seeds in 50 ml of distilled water . It was allowed to stand for 24 h after which it was filtered using a Whitman N. 1 filter paper. The filtrate was directly used as crude extract with 100% concentration. Further dilutions of herbal extracts were made my adding appropriate amount of distilled water.

Characterization and Synthesis Heterocyclic compounds

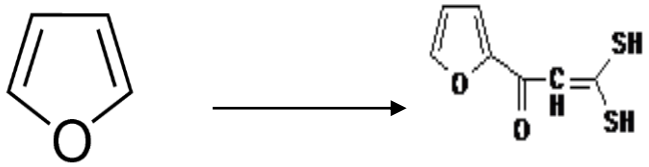
Furan and furan derivate (fig.1) is a class of organic compounds of the heterocyclic aromatic characterized by aring structure composed of one oxygen atoms and four carbon atoms .its colorless, volatile , and somewhat toxic liquid that boils at 33.3 ºC [12] . Furanand related compounds have been reported to possess various biological activities such as antibacterial, antifungal activity [13].



Thiophene 2-cyano-2-sc-thiophene-2-yl-[1,2]dithiol-3ylidene thioactamide

Figure 1 Chemical structure of synthesizes Thiophene derived heterocyclic compounds

Thiophene derivatives (fig.2) have been very well known for their therapeutic applications. Many thiophene derivatives have been developed as chemotherapeutic agents widely used. Thiophene nucleus is one of the most important heterocyclic exhibiting remarkable pharmacological activities [14].



Furan 1-furan-2-yl-thioxo-propenon

Figure 2 Chemical structure of synthesizes furan derived heterocyclic compounds

Microbial Culture

The bacterial , fungal and stryptomyces strains were isoleted from plant diseses such as (potato bacterial soft rot , potato brown rot , potato common scab, tomato fusarium wilt , onion black rot , fruit Rhizopus rot) - faculty of agriculture farm , qena, egypt and used in the present study. The bacterial strains were inoculated into sterile nutrient broth and incubated at 37 °C. Potato dextrose agar (PDA) medium is use for the culturing of F.oxysporium , R.stolonifer and E.cartovora with addition of antibiotic in the medium. Three colonies of bacteria were transferred nutrient Broth. Turbidity of the bacterial suspensions was then adjusted to reach an optical density equivalent to a 0.5 McFarland standard to give a bacterial suspension of 106cfu/mL. Nutrient agar plates were inoculated through streaking bacterial swabs over the entire surface of the plates. Produced Plates were allowed to dry at room temperature and 5 mm disc were input in each plate. In each plate, input one disc in each concentration (20, 40, 80,160 µg/mL solutions of each Fd and Thd derivatives) and (5, 10, 20, 40 µg/mL solutions of each colocynth and cocklebur extracts). Plates were allowed to stand at room temperature to let the tested derivative be diffused into the agar, and afterwards, they were incubated at 37°C for 18 to 24 h. Then, plates were examined for bacterial growth inhibition and zones of inhibition were measured in millimeters.

Evaluation Antimicrobial activity using disc diffusion method

The aqueous extracts were prepared and the sterile blotting paper disc (5 mm) was soaked in the diluted extract in different concentrations. The modified paper disc diffusion was employed to determine the antimicrobial activity of aqueous extract of the herbal preparations [15]. Inoculum was spread over the agar plate using a sterile cotton swab in order to obtain uniform microbial growth. Then the prepared antimicrobial discs were kept over the lawn and pressed slightly along with control. The plates were incubated for 18 h at 37°C. The antimicrobial activity was evaluated and diameter of inhibition zones was measured. Experiment was carried out in triplicate and the averages diameter of zone of inhibition was recorded. The antibacterial activity was classified as highly active (>10 mm), mild active (7-10) mm) and slightly active (6-7 mm) and less than 6 mm was taken as inactive.

Results and Discussion

Antimicrobial activity of synthesis heterocyclic compounds

The impact and the characterization of synthesis compounds on pathogenic plant are summarized in table 1 and the experimental part, respectively. Fd Compound was prepared from reacting Furan with O-CH-S2H2 group; it was added to the set of evaluated derivatives for a comparison purpose. As shown in fig. 3, Furan and all other derivatives were high and mild active against E.cartovora and P.solanacearum. However, some furan compounds showed potency against fungal strain, and some others like furan was totally mild active against streptomyces. Concentration 160 mg/ml showed the highest activity against all fungi strains (zone of inhibition is about 22 mm).

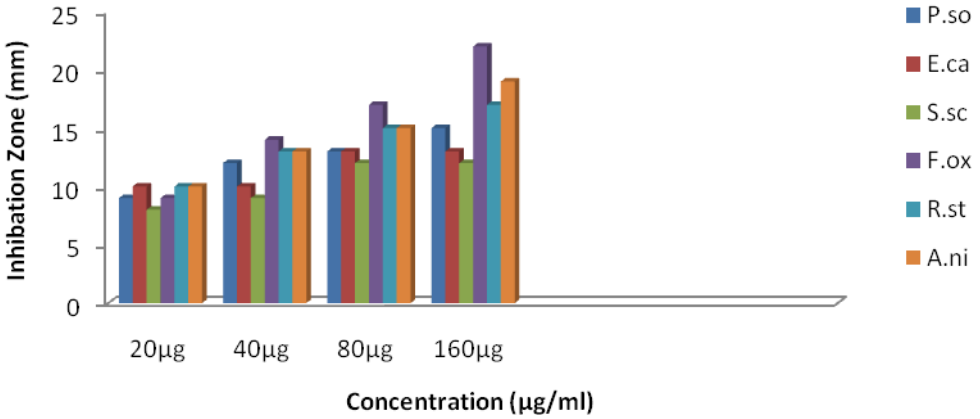


Figure 3 Effect of different furan derivatives concentration on plant pathogen inhibition zone

All prepared thiophene have shown to be susceptible to excellent potency against Gram-negative bacteria P. solanacearum with zone of inhibition ranges from 10 mm to 14 mm. concentration 20mg/ml , showed remarkable activity against P.solancearum with zone of inhibition of about 13 mm and its effect is about two-fold more than that of some other derivatives. Concentration 160 showed even higher potency against A.niger than F.oxsesporum (fig. 4).

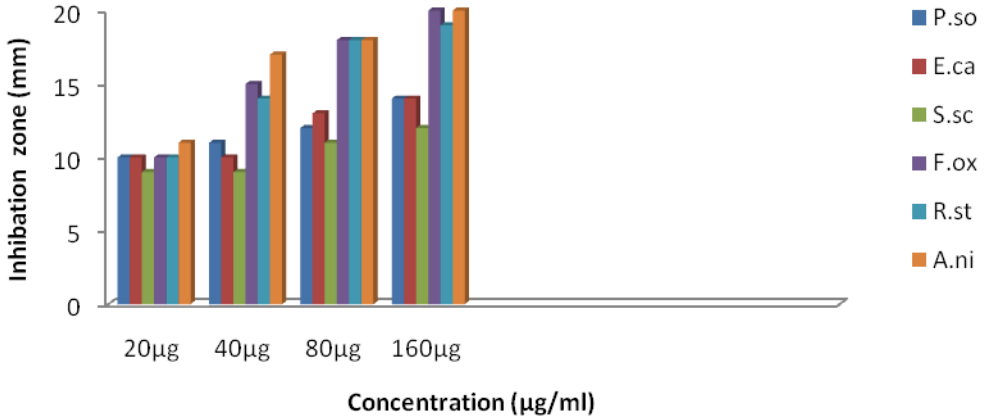


Figure 4 effect of different Thiophene concentration on plant pathogen inhibition zone

These results indicate that furan concentration 80 and 160 have higher activities than concentration 20 and 40. Furan derivate (Fd) has the highest potency against bacterial, fungal strains, which may be attributed to the presence of furan ring. On sequence, thiophene derivates has the highest potency against bacterial, fungal strains, which may be attributed to the presence of Thiophene ring. These results indicate that the improved potency of the prepared compounds could be attributed to the heterocyclic part of the sulfur and oxygen heterocyclic.

Table 1 microbial inhibition zone of synthesized heterocyclic compounds (SHCs)

SHCs	Concentration (Mg/ml)	Microbial Inhibition zone (mm)					
		*F.ox	R.st	A.ni	P.so	E.ca	S.sc
Furan derived	20	9	10	10	9	10	8
	40	14	13	13	12	11	9
	80	17	15	15	13	13	11
	160	22	17	19	15	13	12
Thiophene derived	20	10	10	11	10	10	9
	40	18	14	17	11	10	9
	80	15	18	18	12	13	11
	160	20	19	20	14	14	12

* (F.ox: Fusarium oxysporum , R.st: Rhizopus stolonifer, A.ni: Aspergillius niger, P.so : Pseudomonas solanacearum, E.ca: Erwinia cartovora , S.sc : Streptomyces scabies)

Results revealed that the majority of the synthesized compounds showed varying degree of inhibition against the tested microorganisms. In general, the potency against Gram-negative organisms is greater than against actinomycetes with all concentration (Table 1). All SHCs compounds have the same effect on the isolated microorganisms, because all these compounds contain nearly the same functional groups such as NH2 and CN group. The signed values in the above tables are the most efficient inhibition zones. The following figures (3-4) show inhibition zones resulting from the effect of the tested compounds on the isolated microorganisms at their isolation temperatures.

Antimicrobial activity of herbal extracts (HEs)

The in-vitro antimicrobial activity was performed on three types of bacteria and actinomycetes strains: P. solanacearum , E cartovora and S.scaibes using paper disc diffusion assay . All strains were isolated from plants suffering from bacterial and fungal infections with the relevant bacteria, streptomyces and fungi (fig 8).

Table 2 microbial inhibition zone of herbal aqueous extracts

Herbal extracts	Concentration (Mg/ml)	Microbial Inhibition zone (mm)					
		F.ox*	R.st	A.ni	P.so	E.ca	S.sc
Colocynth	5	10	12	10	11	12	10
	10	16	14	15	14	13	12
	20	18	15	17	15	14	12
	60	21	19	20	18	19	13
Cocklebour	5	12	12	11	13	12	10
	10	14	15	16	14	13	10
	20	18	16	18	14	14	12
	60	21	19	21	17	18	14

* (F.ox: Fusarium oxysporum , R.st: Rhizopus stolonifer, A.ni: Aspergillius niger, P.so : Pseudomonas solanacearum, E.ca: Erwinia cartovora , S.sc : Streptomyces scabies)

The aqueous extract of Colocynthis fruit on two bacteria , streptomyces and three fungal species was depend on the concentration of the extract and the microbial species [16]. The present study revealed that microbial isolates are more sensitive than bacterial isolates toward the aqueous extract of Colocynthis fruits based on the effective concentration .The same results were recorded by Aqil and Ahmed [17] . The inhibitory action of the extract could be attributed to the presence of active compounds in the extract which are water soluble like glucosides and resins which inhibit enzymatic activity in cvtoplasmic membrane [18]. The extract inhibited the growth of all tested fungal and bacterial isolates fig (5), this attributed to the presence of the active compounds and the same recorded by Palanichamy and falix[19].

Figure 5 inhibition zone of different SHCs concentration on bacterial plant pathogen

Colocynthidin and colocynthin alkaloids which may be disrupt cytoplasmic membrane of the microorganisms through their action on lipids and protein [19], furthermore. These compounds may penetrated cytoplasmic membrane and competed the active sites of certain enzymes inside the cell that are essential for multiplication of the microrganisims [20].the extract exerted inhibitory effect on gram-negative bacteria and plant pathogen fungi (fig6) , the present result agree with the study of [21] .which showed that it kills bacteria in respective to their cell wall structure ,however other studies showed that gram-negative bacteria is sensitive [22, 23] . The aqueous extract of cocklebur (X. strumarium) leaves showed different activities against eight pathogenic bacterial strains (fig.7). The biologically active compound

correlated to known substances that possess antibacterial properties [24-27]. The data reported (Table2) presents the antibacterial activity of aqueous extract of leaves was active against 100% bacteria used.

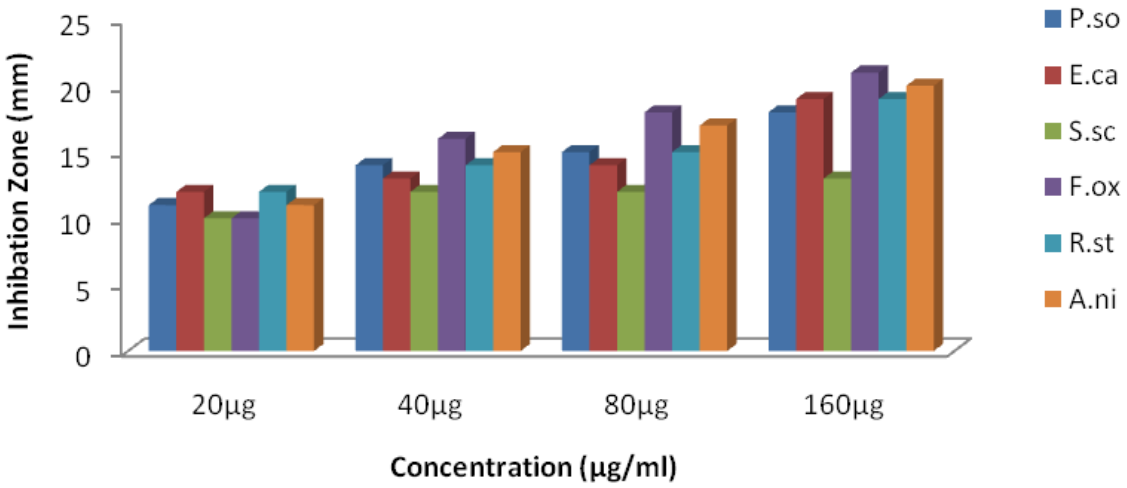


Figure 6 effect of different SHCs concentration on plant pathogen inhibition zone

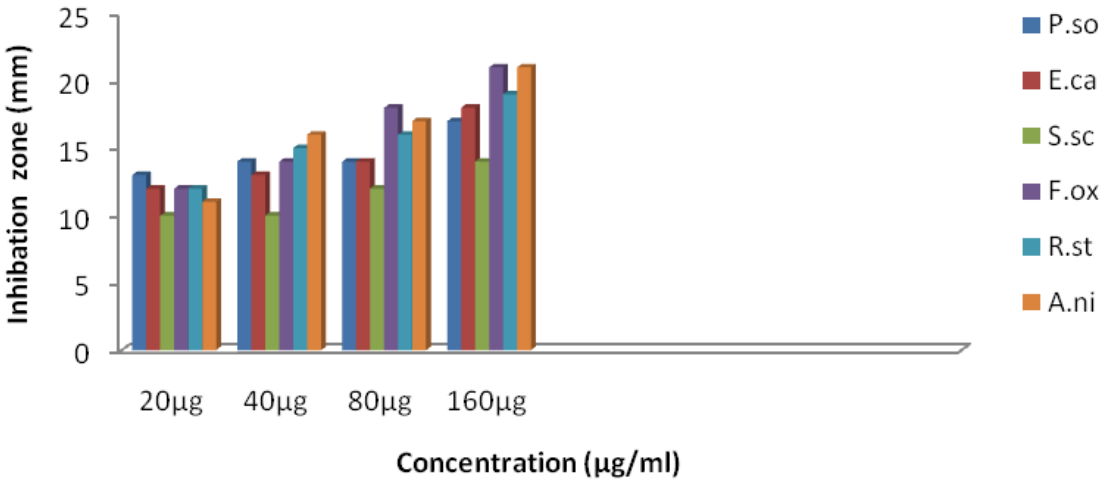


Figure 7 Effect of different cocklebour concentration on plant pathogen inhibition zone

Figure 8 Inhibition zone of different HEs concentration on fungal plant pathogen

Conclusions

Our study suggested that synthesized heterocyclic compounds (SHCs) and herbal extracts (HEs) have great potential as antimicrobial agent against plant pathogen. Hence, it may be recommended that these SHC and two plants could be used in the treatment of plant diseases caused by the above mentioned pathogens. The study also supports the use of these SHCs and HEs not only as the dietary supplement but also as agent to prevent or control the microbial infections.

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