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BIOLOGICALLY ACTIVE COMPOUNDS FROM *LEUCAS LAVANDULAEFOLIA*

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ABSTRACT: The investigation of terrestrial plants origin aimed at searching new natural biologically active compounds is a central issue of this kind of studies, through structure elucidation combined with biological tests. We investigated the biologically active secondary metabolites from *Leucas lavandulaefolia* towards various effects on the behavior of *Aphanomyces cochlioides* zoospores and with antimicrobial activity. We purified the following compounds acacetin, chrysoeriol, luteolin, acacetin 7-O-β-D-glucuronide, acetoside, isoacetoside, salicylic acid and caffeic acid. Their structures were elucidated by spectroscopic analyses and compared with those of the reported data. Five compounds (luteolin, acacetin 7-O-β-D-glucuronide, acetoside, isoacetoside, and salicylic acid) were isolated from *L. lavandulaefolia* for the first time ever. It was found that luteolin showed a strong attractant and encystment activity toward zoospores of the phytopathogenic fungus *A. cochlioides* zoospores. Chrysoeriol also showed an attractant activity towards *A. cochlioides* zoospores. This is the first research report of *L. lavandulaefolia* regarding zoospores bioassay. Antibacterial assay on these compounds indicated that they have antimicrobial activity against the tested bacteria *Bacillus subtilis* and *Staphylococcus aureus*. Some of the identified secondary metabolites provided the evidence for the traditional usage of the plants as different therapeutic potentials (anti-inflammatory, antitussive and anti-diabetes) and may be useful for developing an effective control strategy to contest.

INTRODUCTION: *Leucas lavandulaefolia* Ress. (Family-Labiatae) Syn. *Leucas linifolia* spreng is a well-known traditional medicinal plant widely distributed throughout South East Asia, Africa [1]. It is an herbaceous annual weed, which grows abundantly in fields, pastures, roadsides and waste lands. It is erect, slightly pubescent or tomentose, 0.3 to 0.75 m in height, usually branched; branches are quadrangular, pubescent. Flowers are sub sessile or shortly pedicellate, in axillary and terminal whorls 1.3 to 2 cm diameter 1.3 to 2 cm diameter, toward the end of the branches [2].

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Species of *Leucas* having a characteristic odor are medicinally important plant in Bangladesh and have been extensively used by the rural people from time immemorial for human and cattle ailments [3]. The plants of genus *Leucas* have been used by the tribal in various parts of Asia and Africa. They are used in traditional medicine to cure many diseases such as cough, cold, diarrhea, inflammatory, skin disorder, sedative, vermifuge, stomachic dermatosis [1]. *L. lavandulaefolia* is one of the most common species among genus *Leucas* and this plant as a strong flavour and is eaten as pot-herb.

The leaves are useful as febrifuge and vermifuge. This plant is used as a laxative, anthelmintic, anti-jaundice, asthma, dyspepsia, paralysis [4]. Traditional medicine practitioners prescribe the use of *L. lavandulaefolia* for the treatment of skin diseases,

headache, snake-bite, migraine, loss of appetite, stomach pain, old nervous disorder, old sores and wounds, dermatosis, conjunctivities and antidiabetic agent in traditional system [1, 3, 5].

The phytochemical composition of *L. lavandulaefolia* reported in the literature indicates the presence of acacetin, chrysoeriol, linifoliside, linifoliol, alkaloids triterpenoids, steroids and fatty alcohols in methanol extracts, lupeol and taraxerone [4]. Philomina and Rao identified toxic principals (caffeic acid, chlorogenic acid p-hydroxybenzoic acid, p-coumaric acid, vanillic acid) from the leaves of *L. lavandulaefolia* [6]. Chandrasekhar et al. reported the isolation of *chrysoeriol-6*''(*OAc*)-*4*'-β-*glucoside* from ethanolic extract of the aerial parts of *L. lavandulaefolia* Rees [3].

Aphanomyces cochlioides Drechsler (Saprolegniaceae; Oomycetes), causal agent of the root rot of sugar beet (*Beta vulgaris* L.) and spinach (*Spinacia oleracea* L.) is an oomycete plant pathogen with a facultative necrotrophic growth habit [7-8]. It can survive in the soil as oospores 16-24µm in size; large amounts of oospores are produced in root tissues and is an important concern as it can cause disease in storage facilities of susceptible host crops as well as in living field plants [9]. Warm (~ 78^{o}F) and wet conditions are required for sporangia formation, zoospore release, and germination.

Once these conditions are met, oospores can germinate and produce hyphae that directly infect the host plant, or zoospores can swim through the soil to the roots of the host plant where they encyst, germinate, and infect. The pathogen can survive between crops of appropriate host plants on weed hosts, which can also increase inoculum level in fields where crop rotation is practiced. Plant diseases need to be controlled to preserve the abundance and quality of food, feed, and fiber produced by growers around the world.

To assess the potential role of secondary metabolites in nonhost resistance *L. lavandulaefolia* was tested for the motility behaviour of *A. cochlioides* zoospores. Because this plant extract showed the antimicrobial activity against different microoganisms [4]. Isolation of *A. cochlioides* zoospores regulating principles from nonhost plants may result in interesting compounds for developing an effective control strategy to contest.

Historically, a large number of biologically active secondary metabolites of plant origin have been found to have commercial applications as drugs pesticides, flavors, or as other types of speciality chemicals. Natural products obtained as plant isolates may be useful directly or may serve as starting materials for the synthesis of active agents and/or are suitable as lead compounds for the consequent design of structurally related molecules that are more active or less toxic [10]. There has been an increase in interest in the use of plants with application in traditional medicine as sources of potentially useful compounds. Nevertheless, complicated ways of molecular interactions and bioactivity mechanisms of the extracts or their bioactive constituents provide a challenge to the scientists [11]. Moreover, the increasing use of plant extracts in the food, cosmetic and pharmaceutical industries proposes that, in order to find active compounds, a systematic study of medicinal plants is very significant.

We assessed the potential role of secondary metabolites in non-host resistance, 70% ethanol extracts of *L. lavandulaefolia*, non-host traditional medicinal plant were tested for various effects on the behavior of *A. cochlioides* zoospores using a particle bioassay method for the first time. Based on these we isolated biologically active secondary metabolites from *L. lavandulaefolia* that showed the attractant and halting activity toward *A. cochlioides* zoospores. The antimicrobial activity of this plant extract against different microorganisms has already been reported, although the compounds, which possess antimicrobial activity, have not been identified [4].

Studies on the antimicrobial metabolites also have the possibility of finding new antibiotic compounds. The antimicrobial activity was tested against two Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureas*), one Gram-negative bacterium *Escherichia coli* and a fungus (*Cladosporium herbarum*) and antimicrobial active secondary metabolites were also identified. This paper describes the bioassay-guided identification,

isolation, structure elucidation, and correlation between structures and the activity.

MATERIALS AND METHODS:

General: Merck silica gel 60 F_{254} pre-coated on glass plates was used for analytical and preparative TLC. Column chromatography was conducted using silica gel 60 (spherical, Merck 100-200 mesh). Chemical substances were detected and monitored by a UV detector at 254 nm and 365 nm, I_2 vapors or heating the plates after spraying with 10% H_2SO_4 and spraying with Gibbs reagent, vanillin-H_2SO_4 reagent and thymol reagent.

^1H and ^{13}C NMR spectra were detected on a JEOL JNM-EX 270 FT-NMR spectrometer at 270 MHz using TMS as an internal standard in deuterated chloroform, acetone-d*6*, or methanol-d*4* or DMSO-d*6*. ^1H-^1H COSY, NOSEY, HMQC, and HMBC spectra were recorded on a Bruker AMX-500 spectrometer at 500 MHz. FD-MS and EI-MS spectra were acquired on a JEOL JMS SX-102A and a JEOL DX 500 spectrometer, respectively.

Plant material: The plant material was collected from Jahangirnagar University area, Savar, Dhaka, Bangladesh during Feb-March and verified by Dr. Hadiuzzaman, Faculty of Science, Department of Botany, University of Dhaka. A voucher specimen (DACB Accession No. 28,091) is deposited at the Bangladesh National Herbarium, Mirpur 1, Dhaka 1216.

Culture of *Aphanomyces cochlioides*, preparation of zoospore suspensions, and bioassay: Culture of *A. cochlioides* AC-5 and production of its zoospores were done as described previously [12]. For zoospore assay, we used particle method [13]. Particles of Chromosorb W AW (60/80 mesh) were coated with a test solution/compound solved in EtOAc/acetone at a set concentration dropped into 2 ml of a zoosopore suspension (original zoospore suspension, *ca*. 2 x 10^5/ml, was diluted 2-3 times before each experiment) in a small petri dish (3 cm i. d.). The behavior of zoospores around the particle was observed for several hours after addition of the particles under a microscope (x 53). As a control, particles treated with solvent alone were used.

Antimicrobial assay: As test microbes, two Gram-positive bacteria (*Bacillus subtilis* AHU1036 and *Staphylococcus aureas* AHU1142), one Gram-negative bacterium *Escherichia coli* IFO 3301 and a fungus (*Cladosporium herbarum* AHU9262) were used for antimicrobial test. The antimicrobial activity was evaluated by paper disc method on nutrient-broth agar media [14]. Pentachlorophenol (PCP) and chloramphenicol were used as a positive control. The antifungal activity against *Cladosporium herbarum* was carried out by modified TLC bioautography method [15]. All of the tests were triplicated.

Bioassay guided identification and purification of active compounds: The air-dried stem powdered (1 kg) of *L. lavandulaefolia* was extracted with EtOH (70%) at room temperature for 15 days. The extract was filtered and the solvent was evaporated under reduced pressure to afford a gummy residue (*ca.* 100 g) that showed attracting and halting activity towards *A. cochlioides* zoospores and antimicrobial activity against *B. subtilis*, *S. aureas*, *E. coli* and *C. herbarum.* Subsequently, the extract was fractionated using liquid-liquid partitioning with a separatory funnel between water and *n*-hexane (1:1 v/v) (*ca*. 8.8 g), between water and chloroform (1:1 v/v) (*ca*. 5 g), between water and ethyl acetate (EtOAc) (1:1 v/v) (*ca*. 5.2 g) and finally between water and *n*-butanol (1:1 v/v) (*ca*. 13 g).

After the fractionation, all fractions (*n*-hexane, chloroform, EtOAc and *n*-butanol) were tested in zoospore and antimicrobial bioassay described above. The EtOAc part showed strong attractant and halting activity towards *A. cochlioides* zoospores. $CHCl_3$ soluble part showed weak attractant and halting activity towards *A. cochlioides* zoospores.

So that we further fractionated this two parts. The EtOAc and $CHCl_3$ solubles also showed the antimicrobial activity against *B. subtilis*, *S. aureas*, *E. coli* and *C. herbarum*. We hence chased these compounds too. The aqueous fraction, *n*-butanol fraction and *n*-hexane fraction from this procedure exhibited no activity towards *A. cochlioides* zoospores and antimicrobial activity.

The $CHCl_3$ fraction was chromatographed on silica gel column eluted with *n*-hexane containing increasing amount of EtOAc. Subsequently, all fractions obtained were tested in zoospore and antimicrobial bioassay described above. Fraction 7 obtained with *n*-hexane/EtOAc (100:40 v/v) showed antibacterial activity against *B. subtilis* and *S. aureas* which thus was further purified by crystallization from MeOH furnished compound 1 (acacetin) as light yellowish needles (6 mg). The highest attractant and halting activity towards *A. cochlioides* zoospores was observed in fraction 8 obtained with *n*-hexane/EtOAc (100:50 v/v) which thus was further purified by crystallization from MeOH to yield yellow crystallines (5 mg) of compound 2 (chrysoeriol). Fraction 8 also showed the antibacterial activity against *B. subtilis*, *S. aureas* and *E. coli.*

The EtOAc fraction was fractionated by column chromatography over silica gel eluting with $CHCl_3$/MeOH mixture of increasing polarity. Subsequently, all fractions obtained were tested in zoospore and antimicrobial bioassay described above. Fraction 4 showed the highest attractant and halting activity towards *A. cochlioides* zoospores and antibacterial activity against *B. subtilis*, *S. aureas* and *E. coli.* Fraction 4 obtained with $CHCl_3$/MeOH (90:10 v/v) was subjected to column chromatography (diol silica gel) analysis for active compound identification. Elution was done with an isocratic solvent system. Active fractions (7-9) were obtained with $CHCl_3$/MeOH (25:1 v/v) further crystallized from MeOH furnished compound 3 (luteolin) as a light yellow crystallines (10 mg).

Through PTLC using $CHCl_3$/MeOH/H_2O (65:25:4 v/v/v) as developing solvent, fraction 6 ($CHCl_3$/MeOH, 80:20 v/v) of EtOAc fraction gave flavonoid glycoside (compound 4, acacetin 7-O-β-D-glucuronide, 12 mg) as an inactive compound.
Fraction 10 of EtOAc fraction showed the antibacterial activity against *B. subtilis*, *S. aureas* and *E. coli* which was then fractionated by reverse phase column chromatography. Column was eluted with a mixture of MeOH/H_2O/HCOOH (2:2:0.1 v/v/v) and the focused compounds were contained in fraction 3, which on repeated recrystallization from EtOAc/MeOH mixture gave compound 5 (acetoside, 55 mg) and the mother liquor contained crude 6. Compound 6 was purified by means of preparative TLC (isoacetoside, 4 mg) as an inactive compound.

Fraction 2 of EtOAc fraction eluted with ($CHCl_3$/MeOH 95:5 v/v) showed the antimicrobial activity against *B. subtilis, S. aureas, E. coli and C. herbarum* which was further purified through PTLC using $CHCl_3$/MeOH/H_2O (90:25:3 v/v/v) as the eluent system to yield (5 mg) of salicylic acid (7).

Fraction 5 of EtOAc fraction eluted with ($CHCl_3$/MeOH, 85:15 v/v) showed the antibacterial activity against *B. subtilis*, and *S. aureas* which was further subjected to column chromatography (silica gel column) analysis for active compound identification. Elution was done with an isocratic solvent system of $CHCl_3$/MeOH (20:1 v/v) followed by means of preparative TLC using $CHCl_3$/MeOH/H_2O (95:25:3 v/v/v) as the eluent system to yield yellowish amorphous (7 mg) of compound 8 (caffeic acid).

RESULTS AND DISCUSSION:

Isolation and characterization of zoospore active compounds: Initially, we observed that crude EtOAc soluble showed attractant and halting activity towards *A. cochlioides* zoospores. This observation suggested that *L. lavandulaefolia* release some bioactive compounds. This extract partitioned successively with *n*-hexane, $CHCl_3$, EtOAc and *n*-BuOH. After the fractionation, the EtOAc and $CHCl_3$ soluble part showed attractant and halting activity towards *A. cochlioides* zoospores. The procedures for isolation of zoospore active compounds (compound 2 and compound 3) from *L. lavandulaefolia* were described in the section of Materials and Methods.

As major active compounds causing this activity were identified chrysoeriol (2) and luteolin (3) from $CHCl_3$ and EtOAc soluble part respectively. Their chemical structures were assigned on the basis of physical and spectral data and compared with those of the reported data. The first one from $CHCl_3$ soluble part gave an intense molecular ion peak at *m/z* 300 ($[M]^+$, 100%) in the FD-MS spectrum and analysis of HR-FD-MS established the molecular formula of the compound as $C_{16}H_{12}O_6$. The EI-MS and ^{1}H-NMR data were

found to be reasonably matched with those reported for chrysoeriol (2) [16]. The second one isolated from EtOAc soluble part gave an intense molecular ion peak at *m/z* 286 ($[M]^+$, 100%) in the FD-MS spectrum and analysis of HR-FD-MS established the molecular formula of the compound as $C_{15}H_{10}O_6$. The ^{1}H-NMR data were found to be reasonably matched with those reported for luteolin (3) [17].

Antimicrobial compounds isolated from *L. lavandulaefolia:* Antimicrobial properties of medicinal plants are being increasingly reported from diverse parts of the world. The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional remedies of 80% of the world's population [18]. Antibacterial activity was evaluated by paper disc method using *B. subtilis, S. aureus* and *E.coli* as test bacteria and antifungal activity against *C. herbarium* was evaluated TLC bioautography method.

We isolated five major antibacterial and one antifungal constituent from EtOAc and $CHCl_3$ soluble part of *L. lavandulaefolia.* Chrysoeriol (2) and luteolin (3) isolated as attracting and halting secondary metabolites towards *A. cochlioides* zoospores also showed the antibacterial activity against *B. subtilis, S. aureus* and *E.coli*.

The procedures for isolation of antibacterial compound against *B. subtilis* and *S. aureus* from $CHCl_3$ soluble part of *L. lavandulaefolia* were described in the section of Materials and Methods. The chemical structure was assigned on the basis of physical and spectral data and compared with those of the reported data. An intense molecular ion peak at *m/z* 284 ($[M]^+$, 100%) in the FD-MS spectrum and analysis of HR-FD-MS established the molecular formula of the compound as $C_{16}H_{12}O_5$. The EI-MS and ^{1}H-NMR data were found to be reasonably matched with those reported for acacetin (1) [19].

The other antimicrobial active compounds from EtOAc soluble part was identified as acetoside (=varbascoside) (5), salicylic acid (7) and caffeic acid (8). Compound 5 yellowish amorphous (55 mg) were obtained from EtOAc soluble faction. The structure of compound 5 was confirmed as acetoside by analysis of spectral data and comparison with reported data [20].

Compound 7 colorless needles (5 mg) were obtained from EtOAc soluble faction. An intense molecular ion peak of compound 7 at *m/z* 138 ($[M]^+$, 100%) were observed in the FD-MS spectrum. The FD-HR-MS of compound 7 gave an $[M]^+$ ion at 138.0344 corresponding the molecular formula $C_7H_6O_3$ (calculated 138.0316). The structure of compound 7 was confirmed by analysis of spectral data and direct comparison with authentic sample (salicylic acid) available from our laboratories.

Preliminary studies have shown that the EtOAc soluble part had the antifungal activity against *C. herbariu*. The antifungal activities of all the extracts were evaluated by TLC bioautography using *C. herbariu* as a test fungus. We separated antifungal compounds from the active fraction by PTLC. An intense molecular ion peak of compound 8 at *m/z* 180 ($[M]^+$, 100%) were observed in the FD-MS spectrum. The HR-EI-MS of the compound estimated its molecular formula as $C_9H_8O_4$. This compound was identified as caffeic acid (8) [21]. The structure of compound 8 was also confirmed by direct comparison with authentic sample (caffeic acid) available from our laboratories.

Compound 4 (12 mg, acacetin 7-O-β-D-glucuronide) and compound 6 (4 mg, isoacetoside) light yellow amorphous isolated from EtOAc fraction as an inactive secondary metabolites and identified as acacetin 7-O-β-D-glucuronide and isoacetoside by spectral analyses and compared with reported data respectively [20, 22].

Biological activities of isolated compounds toward the *A. cochlioides* zoospores: The bioactivities of the isolated compounds were evaluated towards the zoospores of *A. cochlioides* (AC-5) in the range of 10^{-2} to 10^{-6} M concentration (**Table 1**). Zoospores of (AC-5) aggregated within a few minutes after chromosorb particles were dropped into the suspension, when the particles had been treated with compounds at the above-mentioned concentrations. The flavonoid luteolin and chrysoeriol showed an attracting and halting activity towards *A. cochlioides* zoospores at a dose 10^{-5} M and 10^{-4} M respectively. The attracting and

halting activity of luteolin was higher than chrysoeriol. Luteolin showed the attractant and halting activity towards *A. cochlioides* zoospores up to the concentration 10^{-5} M. But acacetin did not show any activity towards *A. cochlioides* zoospores.

TABLE 1: ZOOSPORE BIOASSAY OF THE ISOLATED COMPOUNDS TOWARD THE ZOOSPORE OF PHYTOPATHOGENIC FUNGUS *APHANOMYCES COCHLIOIDES**

Compound	Concentration	Effect on *A. cochlioides* zoospores	
		Attractant	Halting
2			
	10^{-5}	-	-
	10^{-4}	+	+
	10^{-3}	++	++
3			
	10^{-6}	+	-
	10^{-5}	++	+
	10^{-4}	+++	++
	10^{-3}	+++	+++
Control		-	-

* In this experiment, Chromasorb W AW particles were coated with a test compound solved in acetone at a set concentration and some particles were dropped into the petri dish (3 cm i.d.) containing 2 ml of a zoospore suspension. The activity was observed around the particles after addition of the particles into the zoospore suspension. `+` sign indicates clear positive bioactivity over control; +++: strong activity; ++: medium activity; +: weak activity; -:non-active. Except compound 2 and 3, others did not show any activity toward the zoospores. Control particles treated with solvent alone. All the tests were triplicated.

The flavonoid acacetin which has fewer hydroxyl groups in the C-ring than luteolin and chrysoeriol, did not show any activity towards *A. cochlioides* zoospores. The hydroxyl group in the C-ring of flavonoid is responsible for that activity. Therefore, it is clear that the activity of the crude EtOAc extract was due to compound luteolin and chrysoeriol. It may be concluded that luteolin play an essential role in the attracting phenomenon.

This is the first report of the attracting and halting activity of luteolin and chysoeiol toward the zoospores *A. cochlioides*. The results of the present work also bring additional data on the zoospore activity of *L. lavandulaefolia.*

Antimicrobial activities of the isolated secondary metabolites from *L. lavandulaefolia*: The antimicrobial activities of the isolated compounds (**Figure 1**) were evaluated against *B. subtilis*, *S. aureus, E. coli* and *C. herbarium* (**Table 2**). The results showed that the notable inhibition of the bacterial growth was shown against the tested organisms. Luteolin demonstrated potent activity against *B. subtilis* and *S. aureus* with MICs 0.13 µg/ml. The antibacterial activity of luteolin was stronger than chrysoeriol and acacetin. The methylation of the hydroxyl group in the B-ring of the flavonoid reduced the activity against the tested microorganisms such as acacetin and chrysoeriol. Chrysoeriol showed the antibacterial activity against *B. subtilis* with MIC 0.14 µg/ml and *S. aureus* with MIC 0.29 µg/ml. All the isolated compounds except salicylic acid were not sensitive against *C. herbarium*.

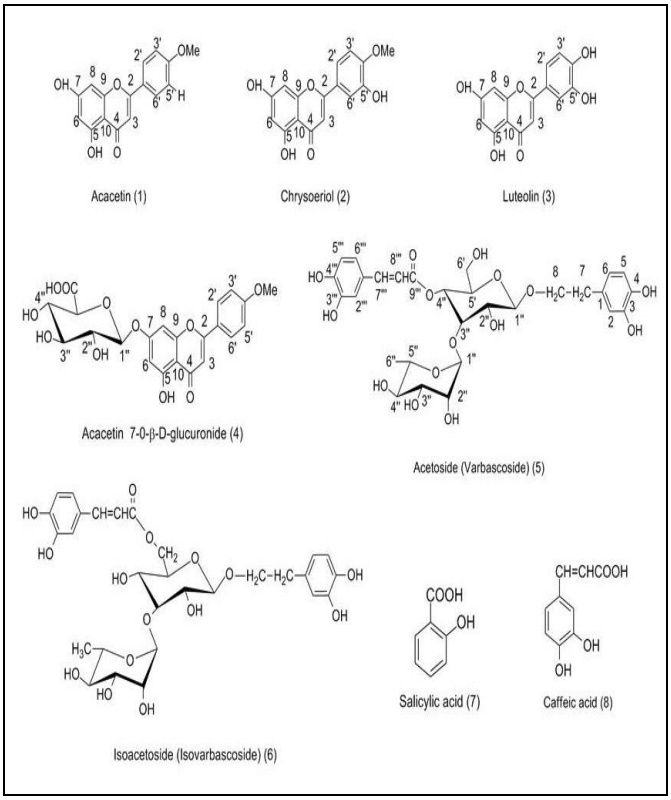

FIGURE 1: SECONDARY METABOLITES ISOLATED FROM *L. LAVANDULAEFOLIA*

The secondary metabolites isolated from *L. lavandulaefolia* exhibited antimicrobial activity that supports folkloric use in the treatment of some diseases as broad-spectrum antimicrobial agents. The presence of flavonoids and phenolic compounds in *L. lavandulaefolia* could account for its antimicrobial activity was in accordance with the results previously obtained [4].

TABLE 2: ANTIMICROBIAL ACTIVITY OF *L. LAVANDULAEFOLIA* ISOLATES AGAINST *BACILLUS SUBTILIS, STAPHYLOCOCCUS AUREAS, ESCHERICHIA COLI* AND *CLADOSPORIUM HERBARUM*

Microbial	Compound	Growth inhibition */dose (µg/disc)				
B. subtilis		**200**	**100**	**50**	**25**	**12.5**
	1	++	+	±	-	-
	2	+++	++	+	±	-
	3	+++	+++	++	+	±
	4	-	-	-	-	-
	5	++	+	+	±	-
	6	-	-	-	-	-
	7	++	++	+	±	-
	8	++	+	+	±	-
	PCP	+++	+++	+++	+++	+++
S. aureas						
	1	+	±	-	-	-
	2	++	+	±	-	-
	3	++	++	±	-	-
	4	NT	NT	NT	NT	NT
	5	++	+	-	-	-
	6	-	-	-	-	-
	7	+	-	-	-	-
	8	+	-	-	-	-
	Chloramphenicol	+++	+++	+++	+++	+++
E. coli						
	1	-	-	-	-	-
	2	+	-	-	-	-
	3	+	-	-	-	-
	4	NT	NT	NT	NT	NT
	5	++	++	-	-	-
	6	-	-	-	-	-
	7	++	++	-	-	-
	8	-	-	-	-	-
	PCP	++	+	+	-	-

*Inhibitory activities are shown owing to the width of the growth inhibitory zone in mm from the edge of a paper disc: +++=>11; ++= 10~5; +=5~1; ±=<1; -= non-inhibition. NT= not tested. Each paper disc (thick type, 8 mm diameter, 1.5 mm thickness, Advantec Toyo, Tokyo) was charged 50 µl of a sample solution containing shown amounts. All of the tests were triplicated. PCP (pentachlorophenol) and chloramphenicol referenced compound. Compound 7 showed the antifungal activity against *C. herbarum* at 20 µg/spot (2 mm). Compounds 1, 2, 3, 4, 5, 6 and 8 did not show any activity against *C. herbarum* up to 40 µg/spot.

Pharmacological properties:

The anti-inflammatory activities of the extract of *L. lavandulaefolia* were reported [4]. The plant extract is employed locally in cough and this could probably be attributed to its antitussive activity [4]. The flavonoid content of the plant could be responsible for the anti-inflammatory activities exhibited by the extract of *L. lavandulaefolia* [23]. The anti-inflammatory and antitussive effect is probably due to the presence of the flavonoids compound acacetin in *L. lavandulaefolia* [24]. Salicylic acid isolated from willow tree is known for its ability to ease aches, pains, reduce fever and it is used as an anti-inflammatory drug [25]. The antioxidant effect of the flavonoids acacetin, chrysoeriol and luteolin had also been reported [26-28]. Plants containing antioxidant principles that have the potential to be developed further for the treatment of large number of major diseases such as cancer, autoimmune disorders, aging, cataract, rheumatoid arthritis, cardiovascular and neurodegenerative diseases [29].

Antimicrobial and antioxidants properties of numerous extracts from many plants have recently been of great interest in both research and the food industry, because their possible use as natural additives emerged from a growing tendency to substitute synthetic antimicrobials and antioxidants with natural ones [30]. Pharmaceutical studies have demonstrated that caffeic acid has many biological effects, such as antibacterial, antioxidant, antiinflammatory, and anticancer growth [31].

The acetoside (verbascoside) and isoacetoside (isoverbascoside) were isolated and identified as biological active metabolites from various plants, most probably linked to the medicinal use of the plant [20, 32-35]. The traditional medicinal use such as the preparation of herbal infusions of aerial parts should result in the extraction of both compounds, since the presence of the sugar moiety renders them water-soluble. Verbascoside, a phenylpropanoid glycoside was obtained from *Buddleia davidii* meristematic cells, attributed for antioxidant, anti-inflammatory and photoprotective actions [32].

The extract of *L. lavandulaefolia* demonstrated hypoglycaemic effect by reduction of blood glucose level [4]. Reduction of blood glucose level could be useful action for the treatment of diabetes. Flavonoid compounds, including luteolin which have been used in treatment of diabetes [36]. Moreover, the presence of luteolin in this plant could probably be responsible for the antidiabetic activity exhibited by the extract.

CONCLUSION: The medicinal plants to be rich in secondary metabolites have been widely used in traditional medicine to fight and cure various illnesses. The present results so far have shown that the flavonoids (chrysoeriol and luteolin) isolated from *L. lavandulaefolia* show attractant activity toward zoospores of *A. cochlioides* zoospores. This supports the effective control strategy to contest the *A. cochlioides* zoospores. The isolated secondary metabolites also possess the antimicrobial activities. The anti-inflammatory, antitussive, anti-diabetes and antioxidant can be attributed to their acacetin, chrysoeriol, luteolin, salicylic acid, caffeic acid and acetoside. Out of eight compounds, five compounds were isolated for the first time from *L. lavandulaefolia*. Further phytochemical and medicinal studies are also required to use their medicinal potentialities.

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