ANTIMICROBIAL ACTIVITIES OF SELECTED THAI MEDICINAL PLANTS BEARING QUINONOIDS

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ฤทธิ์ต้านจุลชีวินจากพืชสมุนไพรไทยบางชนิดที่มีสารกลุ่มควิโนน

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ศึกษาฤทธิ์ต้านจุลชีวินจากพืชสมุนไพรไทย 9 ชนิดที่มีสารกลุ่มควิโนน เพื่อหาความสามารถในการยับยั้งการเจริญเติบโตของเชื้อจุลชีพ สารจากพืชสมุนไพรถูกสกัดด้วยวิธีการหมัก maceration ในปิโตรเลียมอีเทอร์ และเอทานอล ตามลำดับ ทดสอบกับจุลชีพรวม 13 สายพันธุ์ แบ่งเป็นแบคทีเรียชนิดแกรมบวก 5 สายพันธุ์ แบคทีเรียชนิดแกรมลบ 6 สายพันธุ์ และรา 2 สายพันธุ์ มีทั้งชนิดที่ก่อโรคและเชื้อประจำถิ่น ด้วยวิธี agar well diffusion เพื่อดูขอบเขตการยับยั้ง (Inhibition zone) และวิธี broth microdilution เพื่อหาความเข้มข้นต่ำสุดในการยับยั้งการเจริญเติบโต (minimum inhibitory concentration, MIC) และหาความเข้มข้นต่ำสุดที่ไม่พบการเจริญเติบโตของแบคทีเรีย (minimum bactericidal concentration, MBC) หรือความเข้มข้นต่ำสุดที่ไม่พบการเจริญเติบโตของรา (minimum fungicidal concentration, MFC) ผลการศึกษาพบว่าสารสกัดปิโตรเลียมอีเทอร์จากหัวว่านหอมแดง (*Eleutherine americana* (Aubl.) Merr.) แสดงขอบเขตการยับยั้งกว้างที่สุด 32.66±0.58 มม. ต่อเชื้อ *Staphylococcus epidermidis* สารสกัดจากพืชที่มีสารแอนทราควิโนน (anthraquinones) ที่แสดงค่า MIC และ MBC ต่ำสุดคือ สารสกัดปิโตรเลียมอีเทอร์จากรากยอบ้าน (*Morinda citrifolia*) ที่ 125 µg/ml ต่อเชื้อ *Bacillus subtilis* สารสกัดจากพืชที่มีสารเบนโซควิโนน (benzoquinones) ที่แสดงค่า MIC และ MBC ต่ำสุดคือ สารสกัดปิโตรเลียมอีเทอร์จากผลพิลังกาสา (*Ardisia elliptica*) ที่ 62.50 µg/ml ต่อเชื้อ *Bacillus subtilis* และ สารสกัดจากพืชที่มีสารแนฟโทควิโนน (naphthoquinones) ที่แสดงค่า MIC และ MBC ต่ำสุดคือ สารสกัดปิโตรเลียมอีเทอร์จากรากทองพันชั่ง (*Rhinacanthus nasutus*) ที่ 3.90 และ 15.62 µg/ml ตามลำดับ ต่อเชื้อ *Micrococcus luteus* สำหรับสารควิโนนมาตรฐาน จูโกลน (juglone) แสดงขอบเขตการยับยั้งกว้างที่สุด 28.00±2.00 มม. ต่อเชื้อ *Candida albicans* อะลิซาริน (alizarin) ในกลุ่มแอนทราควิโนน: แสดงค่า MIC 100 และ MFC 50 µg/ml ต่อเชื้อ *Candida albicans*, สารเอมบีลิน (embelin) ในกลุ่มเบนโซควิโนน แสดงค่า MIC และ MBC ที่ 6.25 µg/ml ต่อเชื้อ *Bacillus subtilis* and *Bacillus cereus* และ สารลอโซน (lawsone) ในกลุ่มแนฟโทควิโนน แสดงค่า MIC 25 และ MBC 100 µg/ml ต่อเชื้อ *Bacillus cereus* ผลที่ได้จากการทดลองนี้พบว่าสารสกัดพืชที่ได้จากตัวทำละลายทั้ง 2 ชนิด รวมทั้งสารควิโนนมาตรฐานออกฤทธิ์ส่วนใหญ่กับแบคทีเรียชนิดแกรมบวก รา และแบคทีเรียชนิดแกรมลบตามลำดับ สารสกัดปิโตรเลียมอีเทอร์จากว่านหอมแดงและสารสกัดเอทานอลจากกระถินทุ่ง (*Xyris indica*) แสดงฤทธิ์ยับยั้งเชื้อในวงกว้าง ในขณะที่สารสกัดจากชุมเห็ดไทยมีขอบเขตการออกฤทธิ์น้อยที่สุด สารสกัดจากยอทั้งสาม ชนิดแสดงขอบเขตการออกฤทธิ์ที่คล้ายคลึงกัน สารสกัดจากว่านหอมแดงและทองพันชั่งซึ่งเป็นพืชในกลุ่มที่มีสารแนฟโทควิโนนรวมทั้งลอโซนและจูโกลนซึ่งเป็นสารแนฟโทควิโนนมาตรฐานมีความสามารถในการยับยั้งเชื้อดีที่สุด ผลการศึกษานี้สามารถใช้เป็นข้อมูลพื้นฐานของฤทธิ์ต้านจุลชีพที่สนับสนุนสรรพคุณยาของสมุนไพรไทย นำไปสู่การพัฒนาพืชสมุนไพรและภูมิปัญญาการแพทย์แผนไทย

สาขาวิชา วิทยาศาสตร์สาธารณสุข ลายมือชื่อนิสิต .....

ปีการศึกษา 2555 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....

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KEYWORDS : ANTIMICROBIAL ACTIVITY / MEDICINAL PLANT / QUINONES / ANTHRAQUINONES / BENZOQUINONE / NAPHTHOQUINONES

KITTHISAK CHANSUKH: ANTIMICROBIAL ACTIVITIES OF SELECTED THAI MEDICINAL PLANTS BEARING QUINONOIDS. ADVISOR: CHANIDA PALANUVEJ, Ph.D., CO-ADVISOR : ASSOC. PROF. NIJSIRI RUANGRUNGSI, Ph.D., 114 pp.

Antimicrobial activities of selected Thai medicinal plants bearing quinonoids were studied *in vitro*. The crude drugs from nine plant species were sequentially extracted by maceration with petroleum ether and ethanol respectively. Thirteen tested pathogenic and nonpathogenic microorganisms included 5 gram positive bacteria, 6 gram negative bacteria and 2 fungi. The assay was performed by agar well diffusion method for determination of inhibition zone and broth microdilution method for minimum inhibitory concentrations (MIC), minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC) with two fold dilution. The results showed that the petroleum ether extract from *Eleutherine americana* bulbs displayed inhibition zone of 32.66±0.58 mm. against *Staphylococcus epidermidis*. The lowest MIC and MBC for Thai medicinal plants bearing anthraquinones were found in the petroleum ether extract from *Morinda citrifolia* roots that presented MIC and MBC of 125 µg/ml against *Bacillus subtilis. Ardisia elliptica* presenting plant bearing benzoquinones of which the petroleum ether extract from fruits showed the lowest MIC and MBC of 62.50 µg/ml concentration against *Bacillus subtilis*. The plant bearing naphthoquinones, *Rhinacanthus nasutus* roots showed the lowest MIC and MBC of 3.90 and 15.62 µg/ml respectively against *Micrococcus luteus.* Standard quinone derivatives were investigated as well. Juglone displayed inhibition zone of 28.00±2.00 mm. against *Candida albicans.* Alizarin, an anthraquinone compound showed the lowest MIC of 100 and MFC of 50 µg/ml against *Candida albicans*. Embelin, a benzoquinone derivative presented the MIC and MBC of 6.25 µg/ml against *Bacillus subtilis* and *Bacillus cereus*. Lawsone, a naphthoquinone showed the MIC of 25 and MBC of 100 µg/ml against *Bacillus cereus*. Most of the extract and the quinone derivative compounds demonstrated a promising inhibitory effect against gram positive bacteria followed by fungi and gram negative bacteria. *Eleutherine americana* especially petroleum ether extract and *Xyris indica* especially ethanol extract expressed broadest spectrum of antimicrobial activity. *Cassia tora* possessed least spectrum of antimicrobial activity as well as least potency. Three species of *Morinda* showed similar spectrum and potency against tested microorganisms. The extracts from plants bearing naphthoquinones, for example *Eleutherine americana* and *Rhinacanthus nasutus* as well as naphthoquinone compounds, for example lawsone and juglone showed prominent range and potency in antimicrobial activity. This study revealed the antimicrobial potentials among selected Thai medicinal plants bearing quinonoid compounds. The results could expand our knowledge in Thai traditional plant usages and discloses Thai traditional wisdom.

Department : College of Public Health Sciences Student's Signature .....
Field of Study : Public Health Sciences Advisor's Signature .....
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LIST OF ABBREVIATIONS

ATCC	=	American type culture collection
°C	=	Degree celsius
CFU	=	Colony forming unit
cm	=	Centimeter
DMSO	=	Dimethyl sulfoxide
MBC	=	Minimum bactericidal concentration
MFC	=	Minimum fungicidal concentration
MHA	=	Mueller Hinton agar
MHB	=	Mueller Hinton broth
MIC	=	Minimum inhibitory concentrations
mm	=	Millimeter
hrs	=	Hours
ml	=	Milliliter
NA	=	No activity
PGE_2	=	Prostaglandins E_2
SD	=	Standard deviation
SDA	=	Sabouraud Dextrose agar
SDB	=	Sabouraud Dextrose broth
sp.	=	Species
µg	=	Microgram
µl	=	Microliter

CHAPTER I

INTRODUCTION

Background and significance of the study

The use of plants for prevention and treatment of diseases is the earliest type of medicine on earth. The practice of traditional medicine developed along with the cultures of Thailand's ancient times and the others [1]. Historically, pharmacological screening of natural and synthetic compounds have been the source of numerous therapeutic agents. High throughput screening as tool in discovering new biologically active molecules has been most productive in the area of antibiotics. Nowadays, contrary to common belief, drugs derived from higher plants continue to occupy an important niche in modern medicine.

Quinones are the organic compounds in natural. They are presented aromatic compounds form, coloured and contained the same basic chromophore, which can be divided in four groups: anthraquinones, benzoquinones, naphthoquinones and isoprenoid quinones. They are found mainly in bark, roots and tissues of plants [2]. Quinones possess various biological activities, for example, purgative actions from sennosides, anti-microbacterial activity from rhein and saprorthoquinone, antitumor properties from emodin and juglone, prevention of PGE_2 biosynthesis from arnebinone and arnebifuranone and anti-cardiovascular disease from tanshinone [1].

In traditional medicine used plants which have been widely reported that quinones compound for treatment of various diseases such as the leaves, flowers, pods and bark of 3 *Cassia* species: *Cassia sieberiana*, *Cassia alata* and *Cassia occidentalis* (Caesalpiniaceae) are externally applied on itching, cutaneous diseases, fever, ringworm, skin diseases, stomachache, menstrual disorder, headache, hemorrhoids, cholera and diarrhea or dysentery. They are presented anthraquinone derivative contain antimicrobial activity, anticarcinogenicity, antiproliferation and many biological activities [3-8]. In

addition, *Embelia ribes* Burm (Myrsinaceae) has anthelmintic, alterative, tonic, carminative, stomachache and anthelmintic properties. It is useful for intestinal worms, dyspepsia and skin diseases [9]. The plant consists of isolated quinone compound embelin. It is a great herb in worms disturbance. It has the antibacterial and antiprotozoal properties [10]. Furthermore, *Plumbago indica* L. (Plumbaginaceae) is a medicinal plant, long used in traditional medicine. The roots are acrid, astringent and claimed to have thermogenic, anthelmintic, anti-inflammmatory, abortifacient, anti-periodic, carminative, digestive, nerve stimulatory and rejuvenating properties [11]. Plumbagin is a naphthoquinone derivatives which is found in this plant and demonstrated anticancer, antimicrobial, antiprotozoal, anthelmintic, and antifertility activities [12-16].

Each compound from many reports were shown a major investigation on antimicrobial activity. Nevertheless, remaining has a lack of knowledge and scientific study on the antimicrobial potency of plants bearing quinonoids in Thailand. Therefore, this study to evaluate the antimicrobial activities of selected Thai medicinal plants bearing quinonoid.

Objectives of the study

1. To evaluate the antimicrobial activities of selected Thai medicinal plants bearing quinonoid.

2. To compare the antimicrobial potency among each species in selected Thai medicinal plants bearing quinonoid.

Scopes of the study

1. Extraction of Thai medicinal plants bearing quinonoids, namely.

a. *Xyris indica* Linn.	Xyridaceae
b. *Cassia tora* Linn.	Caesalpiniaceae
c. *Morinda elliptica* Ridl.	Rubiaceae

d. *Morinda citrifolia* Linn.	Rubiaceae
e. *Morinda coreia* Ham.	Rubiaceae
f. *Ardisia elliptica* Thunb.	Myrsinaceae
g. *Nigella sativa* Linn.	Ranunculaceae
h. *Rhinacanthus nasutus* (L.) Kurz	Acanthaceae
i. *Eleutherine americana* (Aubl.) Merr.	Iridaceae

2. *In vitro* studies of the antimicrobial activities from extract of Thai medicinal plants bearing quinonoids using susceptibility test with various microorganisms.

a. Determination of zone of inhibition

b. Determination of minimum inhibitory concentrations (MIC)

c. Determination of minimum bactericidal concentration (MBC)

d. Determination of minimum fungicidal concentration (MFC)

3. Comparison of the activity of each microorganism against plant extracts among standard quinone derivatives.

CHAPTER II

LITERATURE REVIEWS

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing is essential aim in the alternative of reasonable agents for therapy. In practice, agents are commonly used empirically and the laboratory test serves to elucidate treatment failures and to give a range of appropriate choice agents. Apart from routine work, antimicrobial susceptibility tests are used to estimate the *in vitro* activity of new agents [17,18]. The protocols that are presented are an extrapolation of methods prepossessing by the Clinical and Laboratory Standards Institute (CLSI) [19].

Agar diffusion method

The method is normal in take action by inoculating a nutrient agar medium in a standardized character and then using the drug to be studied to the agar surface in some type of reservoir. The diffusion of drug is allowed to into the surrounding medium. The test organism displays to a consecutive gradient of drug concentration, with concentration diminishing as duration from the reservoir increase. After incubation of suitable duration, there should be an inhibition zone of organism growth around the reservoir. The size of zone may be measured to determine the degree of susceptibility of test organism [20]. The solution of antimicrobial agents may be applied to surface of a seeded agar medium. Agar disk diffusion method or the Kirby-Bauer method uses filter paper disk that has been moistened with the drug solution and applied directly to the agar while still wet. Disk may be prepared more accurately if a micropipette is used to load each disk with a measured volume of drug solution [21,22]. Alternatively, agar may be cut from the seeded agar medium by Cork borer into specified diameter agar well then the well is filled with the drug solution [23,24].

Broth microdilution method

The broth microdilution method is an adaptation of the broth dilution method using small volumes for routine testing. It utilizes microtiter plastic plates containing 96 wells. The advantage of the system is that it utilizes small volumes of reagents and

allows a large number of bacterium and fungus to be quickly tested [18]. This process is preparing a serial dilution of antibiotics with a liquid growth medium in test wells [25,26]. The wells containing antibiotics are inoculated with a standardized microbial suspension. Following overnight incubation, the wells are examined for visible microbial growth as evidenced by turbidity. The lowest concentration of antibiotics that prevents growth represents the minimal inhibitory concentration (MIC) [27,28]. Simultaneously, the minimum concentration of an agent that kills growth of microorganism is evaluated by sub-culturing from wells showing no turbidity onto antimicrobial agent free media and observing for growth after further incubation. The least concentration with no microbial growth observed on the plate is considered as minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC) value [17].

Quinone

Quinone is a class of organic compounds that are formally derived from aromatic compounds. Quinones are coloured and contain the same basic chromophore, which consists of two carbonyl group in conjugation with two carbon-carbon double bonds. It can conveniently be divided into four groups: benzoquinones, naphthoquinones, anthraquinones and isoprenoid quinones. The three groups are generally hydroxylated, with phenolic properties and may occur *in vivo* either in combined form with sugar as glycoside or in a colourless, sometimes dimeric, quinol form. In such cases, acid hydrolysis is necessary to release the free quinones. The isoprenoid quinones are involved in cellular respiration (ubiquinones) and photosynthesis (plastoquinones) and are thus universally distributed in plants. The prototypical member of the class are 1,4-benzoquinone or cyclohexadienedione, often called simply quinone. Other important examples are 1,2-benzoquinone (ortho-quinone), 1,4-naphthoquinone and 9,10-anthraquinone [2].

Quinone Compounds

Anthraquinones

Anthraquinones are usually substituted with hydroxyl (-OH), hydroxymethyl (-CH_2OH), methoxyl (-OCH_3) and carboxyl (-COOH). Almost anthraquinones have hydroxyl groups in their structures. According to the substituent sites of hydroxyl groups, anthraquinones can be classified into emodin type and alizarin type. The hydroxyl groups in the emodin type of anthraquinones are distributed on two benzene rings, while those in the alizarin type of anthraquinones are only on one benzene ring. Besides the derivatives of anthraquinones, the term anthraquinones also includes anthranol, anthrone and oxidized anthranol. Anthrone can form dimer by linkage at C_{10}-C_{10} (Figure 1) [1]. Alizalin is anthraquinone derivative compound which occurs in the root root of madder plant *Rubia tinctorum* Linn. Rubiaceae (Figure 2) [30]**.**

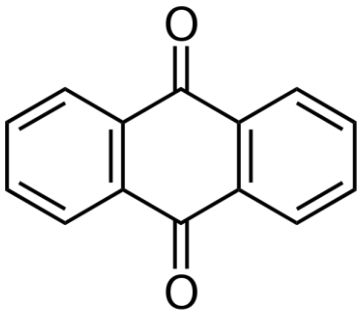


Figure 1 Structures of anthraquinone

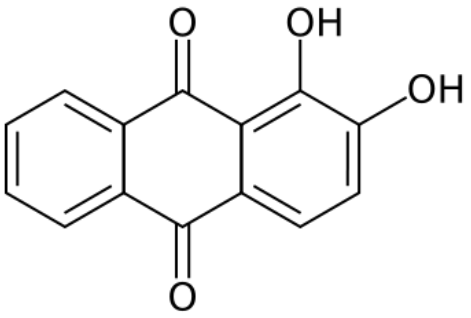
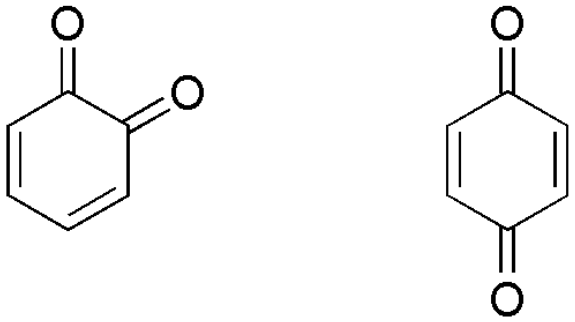


Figure 2 Structures of alizarin (1,2-dihydroxy-9,10-anthracenedione)

Benzoquinones

Benzoquinones are quinones with a single benzene ring, of which there are only two Benzoquinones: 1,4-Benzoquinone, most commonly (also para-benzoquinone, *p*-benzoquinone, para-quinone, or just quinone) and 1,2-Benzoquinone, less commonly (also ortho-benzoquinone, *o*-benzoquinone, ortho-

quinone) (Figure 3) [29]. Benzoquinones are potentially derivable by oxidation of suitable phenolic compounds. Many of these benzoquinones have important biochemical functions in electron transport systems for respiration or photosynthesis. Natural products frequently occur containing the benzoquinone sub-structural unit within the global structure, as can be exemplified by vitamins K_1 and K_2, co-enzyme Q (ubiquinone), and in many terpenes [31**]**. Embelin is benzoquinones which found in fruit of Embelia ribes Burm. Myrsinaceae (Figure 4) [30]**.**



a **b**

Figure 3 Structures of benzoquinones

a. *o*-benzoquinone, **b.** *p*-benzoquinone

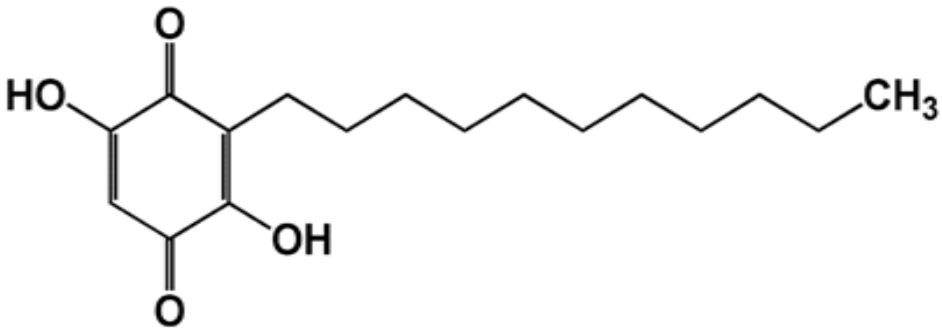


Figure 4 Structures of embelin

(2, 5-dihydroxy-3-undecyl-2, 5-cyclohexadiene-1, 4- benzoquinone)

Naphthoquinones

1,4-Naphthoquinone is an organic compound with formula $C_{10}H_{16}O_2$, which can be viewed as derivatives of naphthalene through the replacement of two hydrogen atoms by two ketone groups. The name is also used generically for two other isomers: 1,2-Naphthoquinone, 2,6-Naphthoquinone (amphi-naphthoquinone). 1,4-Naphthoquinone forms yellow triclinic crystals and has an odor similar to benzoquinone. It is sparingly soluble in cold water, slightly soluble in petroleum ether, and freely soluble in most polar organic solvents. In alkaline solutions, it produces a reddish-brown color. Because of their aromatic stability, 1,4-naphthoquinone derivatives are known to possess anti-bacterial and anti-tumor properties. Naphthoquinone forms the central chemical structure of many natural compounds, most notably the K vitamins. They are mostly found in higher plant, scattered through some twenty families. They have been found in leaves, flowers, woods, bark, roots and fruits (Figure 5) [32,33]. Such as juglone from walnut shells (Figure 6), lapachol derived from the heartwood of a Asian and South American bignoniaceous plants (Figure 7) and lawsone from leaves of *Lawsonia inermis* Linn., *Lawsonia alba* Lam, Lythraceae (Figure 8) [30].

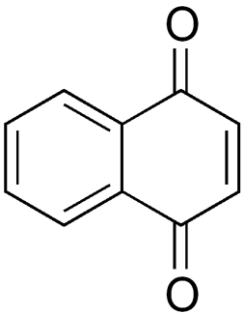


Figure 5 Structures of naphthoquinone

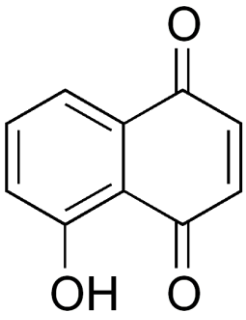


Figure 6 Structures of juglone

(5-hydroxy-1,4-naphthalenedione)

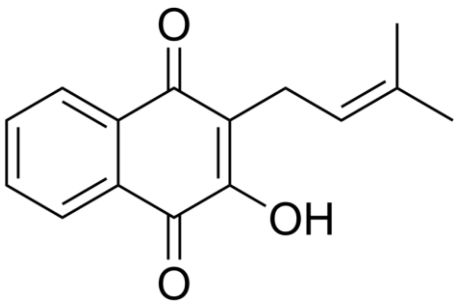


Figure 7 Structures of lapachol

(4-Hydroxy-3-(3-methylbut-2-enyl)naphthalene-1,2-dione)

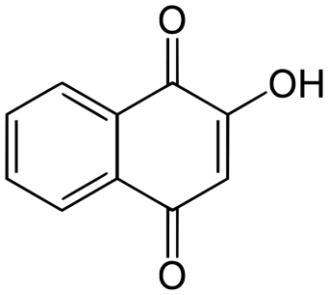


Figure 8 Structures of lawsone

(2-Hydroxy-1,4-naphthoquinone)

Thai medicinal plants bearing quinonoids

***Xyris indica* L.**

Synonyms: *Xyris calocephala* Miquel, *X. capito* Hance, *X. paludosa* R. Brown, *X. robusta* Martius.

Thai name: Kra thin Thung **(**กระถินทุ่ง**)**

Family: XYRIDACEAE

Description of the plant: Herbs perennial, robust. Leaf sheath 7 to 20 or to 25 cm; leaf blade ensiform-linear, 15 to 60 cm×4 to 8 mm, glabrous, veins indistinct when dry, connected by numerous, prominent, trans-verse ribs, apex acute to obtuse. Peduncle 15 to 65 cm, veins prominent. Spikes ovoid, oblong-ovoid, or ellipsoid, 1.2 to 3.5×1 to 1.5 cm; bracts yellowish brown, suborbicular, obovate, or conchiform, 5 to 8×5 to 7 mm, minutely papillose on apical 1/3, base obtuse to truncate, margin gold-colored, membranous, apex entire or emarginate. Lateral sepals linear-spatulate, 5 to 7×0.8 to 1.4 mm, keel denticulate, apex obtuse; median sepal hooded, 4 to 6×2 to 2.5 mm, 1-veined. Petals yellowish to yellow; limb obo-vate to suborbicular, 3 to 4.5×3 to 4 mm, margin serrulate; claw 3.5 to 5× ca. 0.5 mm. Stamens ca. 4 mm; anthers ovate, apex broadly incised, with a short mucro in incision. Staminodes 2 to 3 mm. Ovary

obovoid, 1-loculed. Capsule globose to obovoid, 3 to 4 mm. Seeds ovoid, ca. 0.5 mm. (Figure 9) [34]

Uses: Folklore medicine as a cure for ringworm, itch and leprosy [35,36].

Chemical constituents and pharmacological investigations

Previous study were found three anthraquinones from the flower heads of *Xyris indica* L. namely; chysazin, 3-methoxychrysazin and 3-hydroxy-chrysazin which 3-Hydroxy-chrysazin (1,3,8-trihydroxy-9,10-anthracenedione) is new compounds found in this plant. Antimicrobial evaluation of these three anthraquinones exhibited only marginal or no antibacterial effect at the tested concentrations. However, 3-hydroxy-chrysazin showed a good antifungal activity with MIC values of 0.78 µg/ml against *Trichophyton mentagrophytes* and *Trichophyton rubum*, a dermatophytes causing ringworm and athelete's foot [35]**.**

In addition, the previous study also reported that two isocoumarins named xyridin A and B were isolated from the chloroform extract of the flowering heads of *Xyris indica* Linn. (Xyridaceae). Their structures have been established as 3-n-propyl 1-6,7-(methylenedioxy)-1H-2-benzopyran-1-one and 3-(1'-oxopropy1)-6,7 (methylenedioxy)-1H-2-benzopyran-1-one by means of spectroscopic analyses. Additionally, 3-hydroxy-chrysazin (1,3,8-trihydroxy-9,10-anthracenedione) has been isolated along with chrysazin (1,8-dihydroxy-9,10-anthracenedione), 3-methoxy-chrysazin (1,8-dihydroxy-3-methoxy-9,10-anthracene-dione) and two phytosterols; sigma-spinasterol and stigmasterol [37].

Figure 9 *Xyris indica* Linn. [38]

***Cassia tora* Linn.**

Synonyms: *Senna tora* (L.) Roxb.

Thai name: Chumhet Thai (ชุมเห็ดไทย)

Family: CAESALPINIACEAE

Description of the plant: Herb or undershrub up to 1 m high, nearly glabrous. Leaves with 2 to 4 pairs of leaflets; petioles 1 to 4 cm long; rhachis 2 to 3 with a subulate, about 2 mm long gland between the two lower pairs of leaflets; stipules setaceous, 1 to 5 cm long, more or less canducous. Leafets increasing in size distally with a short petiolule, membranous, obovate; apex broadly rounded, baes cuneate-rounded, 2 to 5 cm long, 1.5 to 2 cm wide. Racemes axillary, short 1 or few-flowered ; bracts linear-acute, 2 to 3 mm long; pedicels 4 to 10 mm (enlarging in fruit); sepals 5, subequal, ovate, about 5 mm long, 2 to 4 mm wide; petals 5, yellow, unequal, obovate, short-clawed with rounded apex, up to 1 cm long, about 6 mm wide; stamens 7 to 10, 3 large, 4 medium, 3 staminodial or absent, rarely perfect; filaments 1.5 to 2 mm long;

anthers 1.5 to 2.5 mm long, opening by apical pores; reduced stamens absent; ovary densely pubescent; style glabrous with truncate apex (stigma). Pods terete, linear, more or falcate, 10 to 15 cm long, about 5 mm wide. Seeds 20 to 30, glossy, brown rhomboidal, 3 to 6 mm long, 2 to 3 mm wide (Figure 10) [39].

Uses: Ayurvedic medicine as a laxative, antiperiodic, useful for leprosy, ringworm, bronchitis and cardiac disorders, ophthalmic, skin diseases, cough, hepatic disorder, liver tonic, haemorroids. [40]

Chemical constituents and pharmacological investigations

Previous study reported that the leaves and seeds of *Cassia tora* contained several types of anthraquinones; chrysophanol, physcion, emodin, rhein, euphol, basseol, obtusifolin, obtusin, chryso-obtusin, rubrofusarin, aurantio-obtusin, chrysophonic acid-9-anthrone including their glycosides and naphthopyrones; rubrofusarin, orrubrofusarin, naptho-alpha-pyrone-toralactune, cassiaside including their glycosides [41-44].

In 2002, Ahmad *et al*. studied on ethanol and aqueous extract from seeds and leaves of *Cassia tora*. The results showed positive effect toward *Candida albicans* with clear inhibition zones of 8.8 mm diameter at 25 mg/ml and 11.1 mm diameter at 30 mg/mL. The ethanol extract of leaves and aqueous extracts of seeds and leaves were not exhibited any distinct inhibition zone. However, the ethanol extract of leaves inhibited the *Microsporum canis* growth while the ethanol extract of seeds showed no effects on this plant. Nevertheless, the ethanol and aqueous extracts of seeds and leaves were not effective against the growth of *Aspergillus fumigates* [45].

Roopashree *et al*., 2008 reported the antibacterial activity from seeds of *Cassia tora.* The aqueous extract from this plant possessed the inhibitory effect against *Staphyllococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* with the concentration of 100 µg/ml, 200 µg/ml and 250 µg/ml, the inhibitory effect on *Bacillus subtilis*. However, the ethanol extract inhibited the growth of *Bacillus subtilis* with the concentration of 64 µg/ml, where as it was not effective against *Staphyllococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. The

methanol extract was effective against both the *Staphyllococcus aureus* and *Escherichia coli* at the concentration of 64 mg/ml but was ineffective against *Pseudomonas areuginosa* and *Escherichia coli.* Therefore, it could be concluded that the aqueous extracts of seeds exhibited the highest antibacterial activity when compared to their petroleum ether, methanol and ethanol extracts in terms of zone of inhibition towards four organisms [46].

Figure 10 *Cassia tora* Linn. [47]

***Morinda elliptica* Ridl.**

Thai name: Yor, Yor Pa, Yor Thuean (ยอ, ยอป่า, ยอเถื่อน)

Family: RUBIACEAE

Description of the plant: It is a small tree usually about 4 to 5 m. tall with white bark. Leaves are narrow-elliptic or oblanceolate shortly acuminate and long narrowed to base. The petioles are 1 cm long with 0.6 cm slender peduncles. Corolla-tubes are white cylindrical with 1 to 1.5 cm long of lobes sub-acute. Fruits are oblong head with green and hardly pulpy in 1 to 1.5 cm long (Figure 11) [48].

Uses: Leaf: eaten to sharpen the appetite, and used in case of fever, headache, cholera, and diarrhea or dysentery. It's the most medicinally used in Malay Peninsula [48].

Chemical constituents and pharmacological investigations

In 1997, Ismail *et al*. isolated 2-formyl-1-hydroxyanthraquinone, 1-hydroxy-2-methylanthraquinone, nordamnacanthal, damnacanthal, lucidin-ω-methyl ether, rubiadin, rubiadin-1-methyl ether, soranjidiol, morindone, morindone-5-methyl ether and alizarin-1-methyl ether from the roots of *Morinda elliptica*. These compounds were investigated for anti-HIV, cytotoxic and antimicrobial activites including *Pseudomonas aeruginosa, Saccharomyces Aspergillus ochraceus, Aspergillus niger* and *Candida lypolitica*. [49, 51]. In addition, the isolation from the leaves and branches was reported a plumieride type iridoid glycloside named morinipticoside [50]. In addition, Chong *et al.* reported that the anthraquinones from *Morinda elliptica* had not only a high availability of antioxidant application but also showed superior antioxidant vitamins levels [52].

Figure 11 *Morinda elliptica* Ridl. [48]

***Morinda citrifolia* Linn.**

Thai name: Yor, Yor Ban (ยอ, ยอบ้าน)

Family RUBIACEAE

Description of the plant: Evergreen shrubs or small trees, to 5 m tall, often fleshy; branches subquadrangular, glabrous. Leaves opposite or solitary opposite an inflorescence; petiole 5 to 20 mm, glabrous; blade fleshy, drying papery, elliptic-oblong, elliptic, or ovate, 10 to 25×5 to 13 cm, glabrous and shiny on both surfaces, base acute oracuminate, apex acute to obtuse; secondary veins 5 to 7 pairs, with pubescent domatia; stipules interpetiolar, free or shortly fused to petioles, broadly triangular to ovate, 4 to16 mm, obtuse or rounded. Inflorescence solitary and leaf-opposed; peduncle 1 to 1.5 cm; head 1, oblong to subglobose, 5 to 10 mm in diam., many flowered; bracts absent. Flowers with hypanthia partially fused, distylous. Calyx glabrous or puberulent; limb subtruncate to truncate, 0.2 to 0.5 mm, sometimes in 1 to numerous flowers of a head with 1 to 3 calycophylls, these white, narrowly elliptic to oblanceolate, 5 to 16 mm, obtuse to acute. Corolla white, funnelform,

outside glabrous; tube ca. 15 mm, densely villous in throat; lobes 5, ovate-lanceolate, ca. 6 mm. Drupecetum white, irregularly ovoid to subglobose, 2.5 to 5 cm. Drupes not distinguishable individually (Figure 12) [53].

Uses: Leaf: infantile diarrhea; decoction with mustard as a favorite domestic remedy, relieve pain; expressed juice of leaves as externally applied to gout, cooling and externally in fever; boiled leaves can applied in fever and headache. Root: emetic and laxative; decoction of roots [48].

Chemical constituents and pharmacological investigations

Previous studies in cell cultures *of Morinda citrifolia* reported that most (more than 90%) of the anthraquinones produced from *Morinda citrifolia* cells are glycosylated especially as -*O*-glucosylxylosyl and subsequently stored in the vacuole [54-57].

In addition, *Morinda citrifolia* roots afforded anthraquinones namely; 2-ethoxy-1-hydroxyanthraquinone,1-hydroxy-2-methylanthraquinone, damnacanthal, nordamnacanthal, 2-formyl-1-hydroxyanthraquinone and morindone-6-methylether. [58].

The other anthraquinone glycosides, for example digiferruginol-1-methylether-11-*O*-β-gentiobioside, digiferruginol-11-*O*-β-primeveroside, damnacanthol-11-*O*-β-primeveroside, 1-methoxy-2-primeverosyloxymethyl-anthraquinone-3-olate, 1-hydroxy-2-primeverosyloxymethyl-anthraquinone-3-olate and 1-hydroxy-5,6-dimethoxy-2-methyl-7-primeverosyloxyanthraquinone) were isolated from *Morinda citrifolia* roots [59].

Many studies investigated the biological activities of *Morinda citrifolia* such as the antibacterial, antiviral, antifungal, antitumor, antihelmintic, analgesic, hypotensive, anti-inflammatory, and immune enhancing effects [60-69].

Several anthraquinone compounds from *Morinda citrifolia* were proved as the antimicrobial agents. In 1950, Bushnel *et al.* reported the antibacterial activities of *Morinda citrifolia* or called Noni which found in Hawaii. The study reported that the

extracts from the ripe of noni fruits exhibited antibacterial activities against *Pseudomonas aeruginosa*, *Micrococcus pyrogenes*, *Escherichia coli*, *Salmonella typhosa*, *Salmonella montevideo*, *Salmonella schottmuelleri* and *Shigella paradys* [70]. In addition, the previous studies also found the antibacterial effect from the roots towards *Pseudomonas aeruginosa, Proteus morgaii, Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Salmonella sp.* and *Shigella sp.* [71,72]. Furthermore, the juice extract from *Morinda citrifolia* fruits also exhibited the antifungal effect on *Candida albicans* [73].

Figure 12 *Morinda citrifolia* Linn. [48]

***Morinda coreia* Ham.**

Synonyms: *Morinda tinctoria* Roxb., *Morinda exserta* Roxb.

Thai name: Yor, Yor Pa, Yor Khon (ยอ, ยอป่า, ยอขน)

Family RUBIACEAE

Description of the plant: A middle-sized tree that usually pubescent or tomentose which spongy bark and deeply cracked grayish yellow. Leaves are not shining and shape as elliptic-obovate or lanceolate, blade 4 to 8 cm with narrowed into petiole 1.3 to 2.5 cm long. Solitary peduncles, axillary or leaf-opposed, and there are frequently in short trichotomous panicles at the end of branchlets. The flowers scented with 5-merous of corolla which are usually tomentose at the outside. There is 1.3 to 2 cm long of tubes with exserted or included of anthers. The syncarpium fruits are 2 cm diameter (Figure 13) [48].

Uses: Root or stem: internally astringent and styptic; the infusion of dries boils is tonic as a bath. Root: stop vomiting in cholera; crushed in alcohol to provide a medicine. Leaf and root: treat ague; a decoction is prescribed and their poultice is applied to the spleen. Bark: astringent and febrifuge [48].

Chemical constituents and pharmacological investigations

Morinda coreia belongs to the *Morinda* species in family Rubiaceae. *Morinda* species are well known for the chemical diversity of anthraquinones, iridoids, saccharide fatty acid esters, and lignans [74]. The major components have been identified in the *Morinda coreia* which includes octoanicacid, potassium, vitamin C, terpenoids, scopoletin, flavonesglycosides, lineoleicacid, anthraquinones, morindone, rubiadin and alizarin [75-77]. In addition, many anthraquinone compounds were isolated from the roots of *Morrinda coreia*. They are nordamnacanthal, damnacanthal, phomarin, 1-methoxy-2-methyl anthraquinone, anthragallol, 1,3,8-trihydroxy-2-methoxy-7-methyl anthraquinone, 1-hydroxy-5,6-dimethoxy-2-methyl anthraquinone and anthraquinone glycoside named 1,3-dihydroxy-2-hydroxymethyl anthraquinone 3-*O*-β-glucopyranosidelucidin-3-*O*-β-glucoside [78].

In 2010, Sivaraman and Muralidharan demonstrated that *Morrinda coreia* possessed antimicrobial and antiinflammatory effects [79]. Previous study also reported that the methanolic extract of *Morrinda coreia* exhibited the antibacterial activities against *Bacillus subtilis, Escherichia coli, Streptococcus aureus, Citrobacter divergens, Klebsiella pnemoniae* and *Aspergillus fumigates* [80]. Moreover, the compounds especially anthraquinones, iridoids, flavonoids also demonstrated a wide range of biological activities including anti-oxidant, anti-malarial, anti-tumor, anti-melanogenesis, anti-diabetic, and chemopreventive activities [74].

Figure 13 *Morinda coreia* Ham. [48]

***Ardisia elliptica* Thunb.**

Synonyms: *Ardisia humilis* Vahl., *Ardisia kotoensis* Hayata, *Ardisia littoralis* Andr., *Ardisia squamulosa* C. Presl., *Bladhia kotoensis* (Hayata) Nakai, *Climacandra obovata* Miq., *Climacandra salicifolia* MIq., *Tinus squamulosa* (C.Presl.) Kuntze

Thai name: Phi Lang Ka Sa (พิลังกาสา)

Family: MYRSINACEAE

Description of the plant: A shrub, up to 5 m tall, with twigs that are swollen at the base and are easily detached. The elliptic to obovate, somewhat fleshy leaves are 2.5 to 5 cm by 8-12 cm and are spirally arranged. At the base they are narrowed and gradually taper to a short, 1 cm long stalk. Flowers measure about 1 cm across, and have five petals and five calyx lobes. They are located in umbell-shaped or condensed clusters located in the axils, with flowers arranged in groups of 8. The calyx has rounded, overlapping lobes, and the pointed petals are white or pink. There are five stamens, and the anthers are many-chambered. Flowers are not fragrant. The ovary is round and has a simple style. The round, few-seeded berry measures about 5 to 12 mm across, first reddish-purple, turning black when ripe. Most commonly recorded as *Ardisia littoralis* in mangrove literature (Figure 14) [81].

Uses: Leaves: Treatment of liver diseases; antidiarrheal; antitussive. Roots: Crushing and steeping inquor, filtrate is taken orally, and remaining portion is made into a poultice, used anti snake venom and anti-VD. Stems: Treatment of leprosy. Flowers: Antimicrobial. Seeds: Treatment of urticaria. Fruits: Antipyretic; antidiarrheal [3].

Chemical constituents and pharmacological investigations

Benzoquinones are able to be produced from the Myrsinaceae family and *Ardisia* genus [82]. The *Ardisia* genus produces several groups of biologically active phytochemicals including saponins, coumarins, and quinines [83]. According to Duke in 2001, the following phytochemical compounds have been isolated from *Ardisia japonica*; 2-hydroxi-5-methoxy-pentadecenyl-benzoquione; ardisin; ardisinol I and II; bergenin; embelin; ilexol; myricitrin; quercetin; quercetrin; and rapanone [84]. In

addition, the distribution of hydroxybenzoquinone derivatives in roots, rhizomes, bark and fruits of eleven species of Myrsinaceae was reported and the presence of embelin, rapanone, maesaquinon, acetylmaesaquinone, 2-hydroxy-5-methoxy-3-pentadecenyl (tridecenyl- and tridecyl-) benzoquinone and ardisiaquinones A, B and C in the plants was established [85,86]. Moreover, the benzoquinone rapanone, the terpenoids bauerenol and amyrin, and the phenolic compounds syringic acid, isorhamnetin, quercetin, bergenin, 5-(Z-Heptadec-4'-enyl)resorcinol and 5-pentadecylresorcinol can be found in *Ardisia elliptica* [87].

Embelin is phytochemical compounds in *Ardisia elliptica*. Previous study demonstrated the potential on antibacterial, anti- inflammatory, antitumor, antioxidation and wound healing [88-94]. In term of antimicrobial, this plant showed the anti-salmonella activity with the minimal inhibitory concentrations (MIC) of the isolated compounds ranged from 15.6 and 125.0 µg/ml [95].

Figure 14 *Ardisia elliptica* Thunb. [96]

***Nigella sativa* Linn.**

Thai name: Thian Dam (เทียนดำ)

Family: RANUNCULACEAE

Description of the plant: Annual herb, 30 to 60 cm high, branching at the top. Stem green, round, hairy, 2 to 5 mm in diameter, internodes 2 to 5 cm long. Leaves alternate, 1 to 3 pinnately dissected into linear, linear-lanceolate, capillary or irregular lobes, lower leaves small, prtioled, upper leaves sessile, 6 to 10 cm long, glabrous on the upper surface, hairy beneath and on the rachis. Flowers regular, bisexual, terminal or axillary on branches, white, greenish white or pale blue, about 3 cm in diameter, long-stalked, pedicels 1.5 to 5.5 cm long becoming longer as the fruit matures. Sepals 5, free, greenish white to pale purple, petaloid, caduceus, lanceolate or ovate 1.2 to 1.5 cm long, 0.4 to 0.5 cm wide, longer than petals. Petals 8, about 5 mm long, about 2.5 mm wide, 3-lobed, anterior lobe, small ovate, acuminate, blue, flapped over the fused concave hairy base of the pair of posterior lobes, posterior lobes, posterior lobes ovate, greenish white, apex blue with a blue line across the body, each carrying a shining green mass, scantily ciliated. Stamens numerous, outer one loner than the inner ones, basifixed; filaments 2.5 to 5.2 mm long, slender; anthers 1.5 to 2 mm long; ovary superior, about 5 mm long, smooth, carpels 2 to 4, styles and stigma about 7 mm long. Fruit united follicles forming a capsule, ultimately inflated with persistent horn-like styles. Seeds ovate to lanceolate, trigonal, black, numerous (Figure 15) [39].

Uses: seeds or its oil are used as a carminative, diuretic, lactagogue and vermifuge, asthma, hypertension, diabetes, inflammation, cough, bronchitis, headache, eczema, fever, dizziness and influenza [97,98].

Chemical constituents and pharmacological investigations

El-Tahir *et al.* 1993 reported that the black seed, *Nigella sativa* in the family Ranunculaceae contained more than 30% of fixed oil and 0.4 to 0.45% w/w of volatile oil. The volatile oil is known to contain 18.4–24% thymoquinone and 46% of monoterpenes such as p-cymene and a-piene [99]. Thymoquinone (2-Isopropropyl-5-

methyl-1, 4-benzoquinone) is one of the most active composition of *Nigella sativa* seed that possesses a good properties including anti-oxidatant and anti-inflamatory activities [100]. In addition, the extracts of the black seeds have many therapeutic effects such as bronchodilatation, immunomodilative, antibacterial, antifungal, hypotensive, hepatoprotective and antidiabetic [101-107].

The methanolic extracts from seed of *Nigella sativa* showed the inhibitory effect on *Staphylococcus aureus* and *Pseudomonas aeruginosa*, but there were inactive on *Klebsiella pneumoiae*, *Escherichia coli* and *Bacillus cereus* [108]. Another research also reported that the oil from *N. sativa* seed showed pronounced dose dependent antibacterial activity which was more against gram positive than gram negative bacteria. Among gram positive bacteria tested, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococci* and *Streptococcus pyogenes* were sensitive to the oil and *Enterococcus faecalis*, *Streptococcus agalactiae* were resistant. For gram negative bacteria tested, only *Pseudomonas aeruginosa* was sensitive to oil while *Acinetobacter baumannii*, *Citrobacter freundii*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Proteus vulgaris* and *Vibrio cholera* were insensitive [109]. In addition, the fixed oil from *Nigella sativa* showed antibacterial activity. The highest activity was found against *Staphylococcus aureus* ATCC 25923 (16.66 mm) and *Salmonella typhimurium* ATCC 14028 (15.33 mm). The best antifungal activity was found against *Candida parapsilosis* ATCC 22019 (13.33 mm) and *Candida glabata* ATCC 90030 (12 mm) [110].

Figure 15 *Nigella sativa* Linn. [111]

***Rhinacanthus nasutus* (L.) Kurz.**

Synonyms: *Justicia nasuta* L., *Rhinacanthus communis* Nees

Thai name: Thong Phan Chang (ทองพันชั่ง)

Family: ACANTHACEAE

Description of the plant: Subshrubs or perennial herbs, to 1.5 m tall. Stems stout, ± 4-angled, faintly striate, densely pubescent when young then glabrescent. Petiole 0.5 to 1.5 cm; leaf blade elliptic, ovate-elliptic, or rarely lanceolate, 2 to 7(-11)×0.8 to 3 cm, abaxially densely pubescent, adaxially sparsely pubescent to subglabrous, secondary veins 5 or 6 on each side of midvein, base cuneate, margin entire or slightly undulate, apex shortly acuminate to acute. Panicles terminal or axillary, to 50 cm; rachis densely pubescent; bracts lanceolate, to 2×0.5 mm; bracteoles ca. 1 mm. Flowers sessile to subsessile. Calyx ca. 5 mm, both surfaces pubescent; lobes

lanceolate, ca. 4×0.7 mm. Corolla greenish white, 2.1 to 2.7 cm, outside pubescent with gland-tipped and non-glandular trichomes; tube 1.5 to 1.8 cm; lower lip 0.75 to 1.2 cm, lobes 2 to 4 mm and subequal; upper lip linear-lanceolate, 6 to 7.5 mm, erect. Staminal filaments glabrous. Style sparsely pubescent. Capsule ca. 2×0.3 cm, pubescent with gland-tipped trichomes. Seeds ca. 2.5 to 2.2 mm, papillose (Figure 16) [112].

Uses: Leaves or barks: Orally, as an antipyretic; treatment of skin diseases, ringworm, hemoptysis; anthelmintic. Roots: Decoction, as an anti-TB; anticancer, antimicrobial; treatment of grey-hair, nail loosing and leprosy [3].

Chemical constituents and pharmacological investigations

Different types of flavonoids, benzenoids, coumarin, anthraquinone, quinone, glycosides, carbohydrate, triterpenes, sterols, anthraquinones and chlorophyll were isolated from the leaves and stems of *Rhinacanthus nasutus*. The significant bioactive ingredients of this plant are known to be naphthoquinones such as rhinacanthin-C, rhinacanthin-D, rhinacanthin-N, rhinacanthin-Q, rhinacanthins (A-D, G-Q), rhinacanthone and lignan groups [113,114]. The isolated compounds from *Rhinacanthus nasutus* named rhinacanthin-C, rhinacanthin-D and rhinacanthin-N possesse antifungal, antibacterial, antiviral, anti-inflammatory and anti-allergic activities as well as the properties for haemorrhoid and various types of cancers treatment [115,116].

Rhinacanthins from *Rhinacanthus nasutus* exhibited potent antifungal activity against *Trichophyton rubrum*, *Trichophyton mentagrophytes*, and *Microsporum gypseum* [117]. In 2010, Puttarak reported that the Rhinacanthins riched in *Rhinacanthus nasutus* extract exhibited potent bactericidal activity against *Streptococcus mutans* with MIC and MBC of 4 µg/ml, and potent bacteriostatic activity against *Staphylococcus epidermidis*, *Propionibacterium acnes* and *Staphylococcus aureus* with the MICs of 8-16 microg/ml. However, the *Rhinacanthus nasutus* extract was not active against *Candida albicans* at concentration up to 2000 µg/ml. The crude plant extract exhibited potent bactericidal activity against gram-positive anaerobic bacteria including *Streptococcus mutans* and *Propionibacterium*

acnes with MBC values of 4 and 32 µg/ml respectively. It also showed moderate bactericidal activity against gram positive aerobic bacteria including *Staphylococcus aureus* and *Staphylococcus epidermidis*, with the MBC values of 256 and 512 µg/ml respectively [118].

Figure 16 *Rhinacanthus nasutus* (L.) Kurz [119]

***Eleutherine americana* (Aubl.) Merr.**

Synonyms: *Eleutherine palmifolia* (Linn.) Merr., *Eleutherine plicata* Herb., *Eleutherine bulbosa* (Mill) Urb., *Sisyrinchium palmifolium* L., *Antholyza meriana* Blanco.

Thai name: Wan Hom Daeng (ว่านหอมแดง)

Family: IRIDACEAE

Description of the plant: The floral sheaths 2 to 10 together in the axils of the 1 to 3 highest cauline leaves; lowermost cauline leaf well-developed, erect, higher ones

bract-like; peduncle erect or more or less patent, often curved or sinuous, 2.25 to 4 cm; floral sheaths 12 to 16 mm long, green, 4-10 flowered; pedicles 1 to 1.5 cm, enclosed within the sheaths; flower ephemerous, inodorous; perianth bright white, 1.5 to 3.5 cm across; tepals oblong, obvate or spatulate, inner ones smaller; anthers and filaments bright yellow; style-arms erecto-patent, alternating with the anthers, yellow. Leaves glabrous, 25 to 60 cm by 1 to 2.5 cm stem erect, obliquely erect drooping or documbent, often sinuous; bulb elongately ovoid (Figure 17) [120].

Uses: bulb was used for carminative and decongestant in children.[121] and treatment of cardiac diseases, especially coronary disorders [122].

Chemical constituents and pharmacological investigations

Previous study found that various naphthoquinones, naphthalene and their derivatives such as hongconin, elecanacin, eleuthoside B, isoeleutherin, eleutherin, eleutherol eleutherinoside A were isolated from *Eleutherine americana* [123-126]. The bioactive compounds presented the antimicrobial, antifungal, antiviral, and antiparasitic properties and also demonstrated the bioactivity as anti-cancer and antioxidant [127-129].

Beatrice *et al.* (2010) investigated antimicrobial activity of crude ethanolic extract of *Eleutherine americana* bulbs towards six gram-positive, seven gram-negative bacteria, six fungal species and two yeasts. The result demonstrated that the ethanolic extract showed a better antibacterial activities against all of gram-positive bacteria compared to gram-negative bacteria. The highest zone inhibition of 20 mm was observed on *Streptococcus* spp., followed by *Bacillus licheniformis*, and the smallest zone inhibition was found on *Bacillus cereus* which produced 13 mm diameter inhibition zone. It was observed that out of eight gram- negative bacteria tested, the extract only demonstrated inhibition against *Erwinia* spp. Additionally the extract demonstrated inhibition against three of the fungi tested: *Aspergillus niger, Rhizopus* spp. and *Penicillium spp*. while no inhibition was observed with yeasts [130]. In addition, this extract showed good antibacterial activity against *Campylobacter* isolates from humans and chicken [131].

Figure 17 *Eleutherine americana* (Aubl.) Merr. [132]

CHAPTER III

MATERIALS AND METHODS

Chemicals

1. Dimethyl sulfoxide (Merck, Germany)
2. Ethanol, Analytical grade (Merck, Germany)
3. Petroleum ether, Analytical grade (Lab-Scan Asia Co., LTD, Thailand)
4. Mueller Hinton agar and broth (Merck, Germany)
5. Sabouraud Dextrose agar and broth (Merck, Germany)
6. Sodium chloride (Mallinckrodt, USA)
7. Alizarin (Sigma, USA)
8. Juglone (Sigma, USA)
9. Lapachol (Sigma, USA)
10. Lawsone (Sigma, USA)
11. Embelin (Chromadex, USA)
12. Ampicllin sodium (T.P. Drug Laboratories (1969) Co., Ltd., Thailand)
13. Amikacin sulfate (T.P. Drug Laboratories (1969) Co., Ltd., Thailand)

Equipments

1. Autoclave (ALP Co., Ltd, Japan)
2. Rotary evaporation (Buchi R210, Switzerland)
3. Hot air oven (WTB binder No.4940006, Germany)

4. Spectrophotometer (T60 Visible Spectrophotometer, PG Instruments Limited, UK)

5. Microtiter plates with 96 wells (Costar, USA)

Plant materials

Plant specimens among 9 species were collected from Thai traditional drug stores, local markets and botanical gardens at different localities of Thailand (Table 1). All of these plants were authenticated by Assoc. Prof. Nijsiri Ruangrungsi, Ph.D. Voucher specimens were deposited at College of Public Health Sciences, Chulalongkorn University, Thailand. Thai medicinal plants bearing quinonoids were studied as follows:

Table 1 Thai medicinal plants bearing quinonoids for antimicrobial activities investigation

Plant	Family	Part used	Collected place
Xyris indica L. (กระถินทุ่ง)	Xyridaceae	flowers	Local market, Bangkok
Cassia tora Linn. (ชุมเห็ดไทย)	Caesalpiniaceae	seeds	Thai traditional drugstore, Bangkok
Morinda elliptica Ridl. (ยอ, ยอป่า, ยอเถื่อน)	Rubiaceae	roots	Botanical garden, Phatthalung
Morinda citrifolia L. (ยอ, ยอบ้าน)	Rubiaceae	roots	Botanical garden, Phatthalung
Morinda coreia Ham. (ยอ, ยอป่า, ยอขน)	Rubiaceae	roots	Botanical garden, Kanchanaburi

Table 1 Thai medicinal plants bearing quinonoids screened for antimicrobial activities. (cont.)

Plant	Family	Part used	Collected place
Ardisia elliptica Thunb. (พิลังกาสา)	Myrsinaceae	fruits	Thai traditional drugstore, Bangkok
Nigella sativa Linn. (เทียนดำ)	Ranunculaceae	seeds	Thai traditional drugstore, Bangkok
Rhinacanthus nasutus (L.) Kurz. (ทองพันชั่ง)	Acanthaceae	roots,	Botanical garden, Bangkok
		aerial parts	Thai traditional drugstore, Bangkok
Eleutherine Americana (Aubl.) Merr. (ว่านหอมแดง)	Iridaceae	bulbs	Thai traditional drugstore, Bangkok

Extraction

The air-dried and powdered plant materials were extracted successively by maceration under room temperature with petroleum ether for 48 hours and finally with 95 % ethanol for 48 hours. The extracts were filtrated and evaporated under reduced pressure at 50 °C by the rotary evaporator to obtain the crude extracts.

The extract yields were weighed, recorded and stored at -20 °C for further antimicrobial testing. The crude extracts were dissolved in DMSO to obtain a concentration of 200 mg/ml for agar diffusion.

Antimicrobial activities testing

Microorganisms

The following microorganisms were used as the tested organisms which included non-spore forming gram-positive bacteria, spore forming gram-positive bacteria, gram-negative bacteria and fungi (Table 2).

Table 2 The tested microorganisms

Tested microorganisms	
Gram positive bacteria (Non-spore forming bacteria)	*Staphylococcus aureus* ATCC6538P[1]
	Micrococcus luteus ATCC9341[2]
	Staphylococcus epidermidis (Isolates)[3]
Gram positive bacteria (Spore forming bacteria)	*Bacillus subtilis* ATCC6633[1]
	Basillus cereus ATCC11778[2]
Gram negative bacteria (Non-spore forming bacteria)	*Escherichia coli* ATCC25922[1]
	Enterobacter aerogenes ATCC13048[2]
	Pseudomonas aeruginosa ATCC9027[1]
	Salmonella typhi (Isolates)[3]
	Salmonella typhimurium ATCC13311[3]
	Shigella spp (Isolates)[3]
Fungi	*Candida albicans* ATCC10230[1]
	Saccharomyces cerevisiae ATCC9763[1]

Source: [1]Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University

[2]Department of Microbiology, Faculty of Sciences and Technology, Suan Sunandha Rajabhat University

[3]Department of Microbiology, Faculty of Sciences, Chulalongkorn University

Preparation of culture media

The bacterial strains were maintained in Mueller Hinton agar (MHA) and Mueller Hinton broth (MHB) for antibacterial testing whereas the fungal strains were maintained in Sabouraud Dextrose agar (SDA) and Sabouraud Dextrose broth (SDB) for antifungal testing.

Preparation of the inoculum

Both bacterial and fungal strains were maintained on Muller-Hinton and Sabouraud agar respectively. They were inoculated at 37 °C, for 18-24 hrs for bacteria and 24-48 hrs for fungi. Four to five of isolated colonies from the overnight culture were suspended in 0.85% of normal saline. The turbidity of the suspension was measured by using the spectrophotometer at 625 nm to obtain the absorbance of 0.08-0.10 which comparable to 0.5 Mc Farland standards. (approximately 1×10^8 CFU/ml) [133-136].

Determination of zone of inhibition

Antimicrobial testing was evaluated by using a slightly modified agar well diffusion method with a two-layer agar technique [137-138]. A 100 µl of the suspension (1×10^8 CFU/ml) was mixed with sterile seeded agar, then poured on the sterile base agar [135]. The plates were allowed to dry at room temperature. Agar wells were cut from seeded agar plates by a cork borer (6 mm.) [23,24, 139].

Twenty microliters of various plant extracts of 200 mg/ml were transferred into each well with diameter of 6 mm. The plates were incubated at 37 °C for 18 to 24 hrs and 24 to 48 hrs for bacterial and fungal strains respectively. The antimicrobial activity was evaluated by measuring the diameters of zone inhibition surrounding the crude extracts. The zones of inhibition were measured in millimeter and the experiment was carried out in triplicates.

Determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

The microdilution method was performed according to Rios and Recio, (2005) [140] with slightly modification. A microbial suspension in broth was prepared by adding 10 µl of normal saline microbial suspensions to 1 ml of Muller-Hinton or Sabouraud broth.

Into a sterile 96-well microplate, 50 µl of microbial suspended in broth was added to the wells containing 50 µl of plant extract (final concentrations: 3.9-2000 µg /ml with two-fold dilution), positive controls (final concentrations: 0.19-100 µg/ml with two-fold dilution) and negative control (DMSO). All of chemicals was prepared by diluting with broth to obtain final volume of 1 ml and incubated at 37^{o} C, for 18 to 24 hrs for bacteria and 24 to 48 hrs for fungi.

The lowest concentration of plant extracts inhibiting the growth of the tested microorganisms detected by the lack of visual turbidity compared to the negative control was defined as the MIC for the extracts [141]. The samples of the known MIC wells were streaked onto nutrient agar plates and incubated at 37^{o} C, for 18-24 hrs for bacteria and 24-48 hrs for fungi. The least concentration with no microbial growth observed on the plate was considered as MBC or MFC value.

CHAPTER IV

RESULTS

Plant extracts

The sequential extractions of nine selected Thai medicinal plants bearing quinonoids were performed by petroleum ether and ethanol respectively. The percent yields were shown in Table 3.

Table 3 Extract yields from Thai medicinal plants bearing quinonoids

Plant	Part used	Yield (% w/w)	
		Petroleum ether	Ethanol
Xyris indica Linn.	Flowers	1.71	6.95
Cassia tora Linn.	Seeds	4.40	4.60
Rhinacanthus nasutus (L.) Kurz	Roots	0.58	2.55
	Aerial parts	0.39	2.32
Morinda elliptica Ridl.	Roots	0.40	7.13
Morinda citrifolia Linn.	Roots	0.36	8.74
Morinda coreia Ham.	Roots	0.75	5.42
Ardisia elliptica Thunb.	Fruits	1.28	6.91
Eleutherine americana (Aubl.) Merr.	Bulbs	0.95	6.63
Nigella sativa Linn.	Seeds	18.85	6.14

Antimicrobial activities

The antibacterial and antifungal potential among Thai medicinal plants bearing quinonoids were determined *in vitro*. Eleven bacteria including 5 species of gram-positive bacteria and 6 species of gram-negative bacteria were tested. Two species of fungi were also used for the studies.

Twenty extracts from 9 Thai medicinal plants bearing quinonoids, as well as standard quinone derivatives and antibiotic drugs were firstly tested by agar well diffusion method against 13 species of microorganisms mentioned above. The extracts and chemicals presenting inhibition zones were further evaluated for MIC, MBC and MFC respectively.

Most of the extracts and chemiclas showed the inhibitory activity against *Basillus cereus* except for the petroleum ether and ethanol extracts from *Cassia tora* seeds, the ethanol extract from *Rhinacanthus nasutus* roots and juglone. The petroleum ether extract from *Rhinacanthus nasutus* demonstrated the lowest MIC and MBC among the studied plants. The selected plants and standard quinone derivatives presenting high potential against *Basillus cereus* were as follows: *Rhinacanthus nasutus* roots (MIC and MBC =15.62 µg/ml), *Ardisia elliptica* fruits (MIC=62.50 and MBC=125 µg/ml), *Eleutherine americana* bulbs (MIC=62.50 and MBC=125 µg/ml), and embelin (MIC and MBC=6.25 µg/ml). The results were demonstrated in Table 4.

Table 4 Antimicrobial activity against *Basillus cereus* among the extracts from selected plants bearing quinonoids and standard quinone derivatives

Samples	Part used	Solvent extracts	*Basillus cereus*		
			Inhibition zone (mm)	MIC (µg/ml)	MBC (µg/ml)
Plants					
Xyris indica	Flowers	Petroleum ether	7.33±0.58	250	2000
		Ethanol	13.33±0.58	500	2000
Cassia tora	Seeds	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Rhinacanthus nasutus	Roots	Petroleum ether	19.67±0.58	15.62	15.62
		Ethanol	NA	NA	NA
	Aerial parts	Petroleum ether	10.67±0.58	500	1000
		Ethanol	12..67±0.58	500	1000
Morinda elliptica	Roots	Petroleum ether	12.00±1.00	250	500
		Ethanol	10.33±0.58	500	2000
Morinda citrifolia	Roots	Petroleum ether	15.33±0.58	125	250
		Ethanol	15.00±1.00	500	1000
Morinda coreia	Roots	Petroleum ether	15. 67±0.58	500	1000
		Ethanol	9.67±0.58	500	2000
Ardisia elliptica	Fruits	Petroleum ether	7.33±0.58	62.50	125
		Ethanol	9.33±0.58	250	500
Eleutherine americana	Bulbs	Petroleum ether	24.33±0.58	62.50	125
		Ethanol	20.00±0.00	125	250
Nigella sativa	Seeds	Petroleum ether	18.33±0.58	250	250
		Ethanol	15.00±0.00	2000	2000
Standard quinone derivatives					
Alizarin			10.33±0.58	25	>100
Juglone			NA	NA	NA
Lapachol			14. 67±0.58	50	>100
Lawsone			15.33±0.58	25	100
Embelin			9. 67±0.58	6.25	6.25
Positive controls					
Ampicllin sodium			40.33±0.58	0.19	0.19
Amikacin sulfate			32.00±0.00	0.39	0.39
Negative control					
DMSO			NA	NA	NA

Means ± SD, NA = no activity, diameter of well = 6 mm. The tests were done in triplicate.

For the inhibitory activity against *Bacillus subtilis*, the extracts and standard quinone derivatives exhibited antimicrobial property except for ethanol extract from *Cassia tora* seeds. The petroleum ether extracts of *Rhinacanthus nasutus* roots and *Ardisia elliptica* fruits presented potent activity according to MIC of 7.81 µg/ml and MBC of 15.62 µg/ml for *Rhinacanthus nasutus* and MIC as well as MBC of 62.50 µg/ml for *Ardisia elliptica*. For standard quinone derivatives, embelin showed the lowest MIC and MBC of 6.25 µg/ml. The results were shown in Table 5.

Table 5 Antimicrobial activity against *Basillus subtilis* among the extracts from selected plants bearing quinonoids and standard quinone derivatives

Samples	Parts used	Solvent extracts	*Bacillus subtilis*		
			Inhibition zone (mm)	MIC (µg/ml)	MBC (µg/ml)
Plants					
Xyris indica	Flowers	Petroleum ether	7.33±0.58	250	1000
		Ethanol	12.33±0.58	500	2000
Cassia tora	Seeds	Petroleum ether	8.33±1.50	>2000	>2000
		Ethanol	NA	NA	NA
Rhinacanthus nasutus	Roots	Petroleum ether	16.00±0.00	7.81	15.62
		Ethanol	12.67±0.58	125	125
	Aerial parts	Petroleum ether	14.67±0.58	250	250
		Ethanol	13.00±1.00	250	500
Morinda elliptica	Roots	Petroleum ether	12.00±1.00	250	250
		Ethanol	11.00±1.00	500	1000
Morinda citrifolia	Roots	Petroleum ether	14.33±0.58	125	125
		Ethanol	16.67±0.58	250	250
Morinda coreia	Roots	Petroleum ether	14.67±0.58	250	250
		Ethanol	12.67±0.58	250	1000
Ardisia elliptica	Fruits	Petroleum ether	7.00±0.00	62.50	62.50
		Ethanol	9.67±0.58	250	250
Eleutherine americana	Bulbs	Petroleum ether	17.67±0.58	125	125
		Ethanol	15.67±0.58	250	500
Nigella sativa	Seeds	Petroleum ether	12.00±0.00	125	125
		Ethanol	10.00±0.00	2000	2000
Standard quinone derivatives					
Alizarin			11.00±0.00	25	>100
Juglone			11.00±0.00	25	>100
Lapachol			10.00±1.00	100	>100
Lawsone			25.67±1.20	50	>100
Embelin			10.00±0.00	6.25	6.25
Positive controls					
Ampicllin sodium			19.33±0.58	12.50	12.5
Amikacin sulfate			29.33±0.58	0.19	0.39
Negative control					
DMSO			NA	NA	NA

Means ± SD, NA = no activity, diameter of well = 6 mm. The tests were done in triplicate.

Most of the extracts and standard quinone derivatives exhibited *Staphylococcus aureus* inhibition except for the petroleum ether extract of *Xyris indica* flowers, both extracts of *Cassia tora* seeds and petroleum ether extract of *Ardisia elliptica* fruits. The extracts from *Rhinacanthus nasutus* roots showed potent growth inhibition (MIC 7.81 and 62.50 µg/ml for petroleum ether and ethanol extracts respectively) but weak bacteriocidal activity against *Staphylococcus aureus* (MBC > 2,000 µg/ml). The potential activities according to MIC and MBC were from the ethanol extract of

Ardisia elliptica fruits (31.25 and 250 µg/ml respectively) and the petroleum ether extract of *Eleutherine americana* bulb (62.50 and 500 µg/ml respectively). For standard quinone derivatives, embelin showed the lowest MIC and MBC of 6.25 and 12.50 µg/ml respectively. The results were shown in Table 6.

Table 6 Antimicrobial activity against derivatives *Staphylococcus aureus* among the extracts from selected plants bearing quinonoids and standard quinone derivatives

Samples	Parts used	Solvent extracts	*Staphylococcus aureus*		
			Inhibition zone (mm)	MIC (µg/ml)	MBC (µg/ml)
Plants					
Xyris indica	Flowers	Petroleum ether	NA	NA	NA
		Ethanol	10.33±0.58	125	>2000
Cassia tora	Seeds	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Rhinacanthus nasutus	Roots	Petroleum ether	16.00±0.00	7.81	>2000
		Ethanol	11.00±1.00	62.50	>2000
	Aerial parts	Petroleum ether	11.67±0.58	500	>2000
		Ethanol	11.33±0.58	500	2000
Morinda elliptica	Roots	Petroleum ether	11.33±0.58	125	>2000
		Ethanol	11.00±0.00	250	>2000
Morinda citrifolia	Roots	Petroleum ether	11.67±0.58	125	>2000
		Ethanol	12.00±0.00	125	2000
Morinda coreia	Roots	Petroleum ether	12.00±0.00	125	>2000
		Ethanol	10.33±0.58	125	>2000
Ardisia elliptica	Fruits	Petroleum ether	NA	NA	NA
		Ethanol	8.33±0.58	31.25	250
Eleutherine americana	Bulbs	Petroleum ether	21.00±0.00	62.50	500
		Ethanol	15.00±0.00	125	2000
Nigella sativa	Seeds	Petroleum ether	10.67±0.58	250	500
		Ethanol	8.67±0.58	500	>2000
Standard quinone derivatives					
Alizarin			12.67±0. 0.58	12.50	>100
Juglone			20.00±0.00	12.50	>100
Lapachol			10.00±0.00	50	>100
Lawsone			20.00±0.00	12.50	>100
Embelin			10.00±0.00	6.25	12.50
Positive controls					
Ampicllin sodium			50.33±0.58	0.19	6.25
Amikacin sulfate			30.00±0.00	1.56	3.12
Negative control					
DMSO			NA	NA	NA

Means ± SD, NA = no activity, diameter of well = 6 mm. The tests were done in triplicate.

For *Staphylococcus epidermidis* inhibition, the petroleum ether extracts of *Rhinacanthus nasutus* roots and *Xyris indica* flowers showed the value of MIC at

15.62 and 62.50 µg/ml respectively. Alizarin was the only standard quinone derivative which had no activity against *Staphylococcus epidermidis*. Embelin showed strongest antibacterial activity against *Staphylococcus epidermidis* with the MIC and MBC of 12.50 and 50 µg/ml respectively. The results were shown in Table 7.

Table 7 Antimicrobial activity against derivatives *Staphylococcus epidermidis* among the extracts from selected plants bearing quinonoids and standard quinone derivatives

Samples	Parts used	Solvent extracts	*Staphylococcus epidermidis*		
			Inhibition zone (mm)	MIC (µg/ml)	MBC (µg/ml)
Plants					
Xyris indica	Flowers	Petroleum ether	11.00±1.00	62.50	>2000
		Ethanol	16.00±1.00	500	>2000
Cassia tora	Seeds	Petroleum ether	NA	NA	NA
		Ethanol	7.33±0.58	2000	>2000
Rhinacanthus nasutus	Roots	Petroleum ether	29.33±0.58	15.62	>2000
		Ethanol	15.33±0.58	125	>2000
	Aerial parts	Petroleum ether	10.00±0.00	>2000	>2000
		Ethanol	13.00±0.00	>2000	>2000
Morinda elliptica	Roots	Petroleum ether	14.66±0.58	250	>2000
		Ethanol	10.66±0.58	500	>2000
Morinda citrifolia	Roots	Petroleum ether	16.33±0.58	250	1000
		Ethanol	16.66±0.58	500	>2000
Morinda coreia	Roots	Petroleum ether	21.00±1.00	250	1000
		Ethanol	11.33±0.58	1000	>2000
Ardisia elliptica	Fruits	Petroleum ether	7.00±0.00	125	500
		Ethanol	10.66±0.58	1000	2000
Eleutherine americana	Bulbs	Petroleum ether	32.66±0.58	125	>2000
		Ethanol	20.00±0.00	500	>2000
Nigella sativa	Seeds	Petroleum ether	15.33±0.58	2000	>2000
		Ethanol	10.33±0.58	>2000	>2000
Standard quinone derivatives					
Alizarin			NA	NA	NA
Juglone			20.66±0.58	1.56	>100
Lapachol			25.00±0.00	25	>100
Lawsone			22.66±0.58	50	>100
Embelin			7.00±0.00	12.50	50
Positive controls					
Ampicllin sodium			30.66±0.58	0.39	0.78
Amikacin sulfate			31.33±0.58	0.78	1.56
Negative control					
DMSO			NA	NA	NA

Means ± SD, NA = no activity, diameter of well = 6 mm. The tests were done in triplicate.

Both extracts of *Cassia tora* seeds and the petroleum ether extract of *Xyris indica* flowers showed no inhibitory action against *Micrococcus luteus*. *Rhinacanthus nasutus* roots exhibited potent inhibition against *Micrococcus luteus*. The petroleum

ether extract from *Rhinacanthus nasutus* roots showed the lowest MIC of 3.90 µg/ml and MBC of 15.62 µg/ml compared to the ethanol extract MIC and MBC of 62.50 µg/ml. For standard quinone derivatives, embelin showed the highest potency (MIC= 6.25 and MBC=12.50 µg/ml). The results were shown in Table 8.

Table 8 Antimicrobial activity against derivatives *Micrococcus luteus* among the extracts from selected plants bearing quinonoids and standard quinone derivatives

Samples	Parts used	Solvent extracts	*Micrococcus luteus*		
			Inhibition zone (mm)	MIC (µg/ml)	MBC (µg/ml)
Plants					
Xyris indica	Flowers	Petroleum ether	NA	NA	NA
		Ethanol	11.33±0.58	500	>2000
Cassia tora	Seeds	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Rhinacanthus nasutus	Roots	Petroleum ether	23.33±0.58	3.90	15.62
		Ethanol	22.00±0.00	62.50	62.50
	Aerial parts	Petroleum ether	19.00±1.00	500	2000
		Ethanol	16.33±0.58	500	2000
Morinda elliptica	Roots	Petroleum ether	9.00±1.00	250	1000
		Ethanol	12.00±1.00	500	>2000
Morinda citrifolia	Roots	Petroleum ether	15.00±0.00	125	250
		Ethanol	15.66±0.58	125	2000
Morinda coreia	Roots	Petroleum ether	18.00±1.00	125	>2000
		Ethanol	11.00±0.00	500	>2000
Ardisia elliptica	Fruits	Petroleum ether	9.66±0.58	125	125
		Ethanol	11.00±1.00	125	250
Eleutherine americana	Bulbs	Petroleum ether	20.00±0.00	125	500
		Ethanol	14.66±0.58	2000	>2000
Nigella sativa	Seeds	Petroleum ether	15.00±0.00	>2000	>2000
		Ethanol	9.66±0.58	>2000	>2000
Standard quinone derivatives					
Alizarin			NA	NA	NA
Juglone			22.00±1.00	6.25	>100
Lapachol			NA	NA	NA
Lawsone			15.00±0.00	50	>100
Embelin			9.66±0.58	6.25	12.50
Positive controls					
Ampicllin sodium			46.00±1.00	0.19	0.39
Amikacin sulfate			30.00±0.00	0.78	1.56
Negative control					
DMSO			NA	NA	NA

Means ± SD, NA = no activity, diameter of well = 6 mm. The tests were done in triplicate.

Only 4 from 20 extracts of selected plant materials showed antibacterial activity against *Escherichia coli*. These were the ethanol extracts of *Xyris indica* flowers, *Rhinacanthus nasutus* roots, *Ardisia elliptica* fruits and the petroleum ether extract of

Cassia tora. However, their MIC and MBC of 2,000 µg/ml or more showed those of marginal potency. For standard quinone derivatives, lapachol and embelin had no inhibitory effect. Alizarin, juglone and lawsone presented *Escherichia coli* inhibition potential with the MIC of 25 µg/ml and MBC >100 µg/ml. The results were shown in Table 9.

Table 9 Antimicrobial activity against derivatives *Escherichia coli* among the extracts from selected plants bearing quinonoids and standard quinone derivatives

Samples	Parts used	Solvent extracts	*Escherichia coli*		
			Inhibition zone (mm)	MIC (µg/ml)	MBC (µg/ml)
Plants					
Xyris indica	Flowers	Petroleum ether	NA	NA	NA
		Ethanol	9.67±0.58	2000	>2000
Cassia tora	Seeds	Petroleum ether	9.00±1.00	>2000	>2000
		Ethanol	NA	NA	NA
Rhinacanthus nasutus	Roots	Petroleum ether	NA	NA	NA
		Ethanol	10.33±0.58	>2000	>2000
	Aerial parts	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Morinda elliptica	Roots	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Morinda citrifolia	Roots	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Morinda coreia	Roots	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Ardisia elliptica	Fruits	Petroleum ether	NA	NA	NA
		Ethanol	10.00±1.00	>2000	>2000
Eleutherine americana	Bulbs	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Nigella sativa	Seeds	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Standard quinone derivatives					
Alizarin			11.67±0.58	25	>100
Juglone			13.33±0.58	25	>100
Lapachol			NA	NA	NA
Lawsone			19.33±0.58	25	>100
Embelin			NA	NA	NA
Positive controls					
Ampicllin sodium			32.00±0.00	3.12	3.12
Amikacin sulfate			21.00±0.00	0.78	1.56
Negative control					
DMSO			NA	NA	NA

Means ± SD, NA = no activity, diameter of well = 6 mm. The tests were done in triplicate.

Only two extracts possessed the inhibitory activity against *Enterobacter aerogenes* namely the ethanol extract from *Xyris indica* flowers and the petroleum

ether extract from *Eleutherine americana* bulbs. They presented the inhibition zone of 8.67±0.58 mm, MIC and MBC >2000 µg/ml. Five standard quinone derivatives were not active on this microbe. The results were shown in Table 10.

Table 10 Antimicrobial activity against derivatives *Enterobacter aerogenes* among the extracts from selected plants bearing quinonoids and standard quinone derivatives

Samples	Parts used	Solvent extracts	*Enterobacter aerogenes*		
			Inhibition zone (mm)	MIC (µg/ml)	MBC (µg/ml)
Plants					
Xyris indica	Flowers	Petroleum ether	NA	NA	NA
		Ethanol	8.67±0.58	>2000	>2000
Cassia tora	Seeds	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Rhinacanthus nasutus	Roots	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
	Aerial parts	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Morinda elliptica	Roots	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Morinda citrifolia	Roots	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Morinda coreia	Roots	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Ardisia elliptica	Fruits	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Eleutherine americana	Bulbs	Petroleum ether	8.67±0.58	>2000	>2000
		Ethanol	NA	NA	NA
Nigella sativa	Seeds	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Standard quinone derivatives					
Alizarin			NA	NA	NA
Juglone			NA	NA	NA
Lapachol			NA	NA	NA
Lawsone			NA	NA	NA
Embelin			NA	NA	NA
Positive controls					
Ampicllin sodium			18.66±0.58	50	100
Amikacin sulfate			23.33±0.58	0.78	1.56
Negative control					
DMSO			NA	NA	NA

Means ± SD, NA = no activity, diameter of well = 6 mm. The tests were done in triplicate.

All extracts and standard quinone derivatives except lawsone were not active against *Pseudomonas aeruginosa* and *Salmonella typhi*. Lawsone showed the inhibition zone of 12.67±0.58 mm, MIC and MBC > 100 µg/ml. The results were shown in Table 11 and 12.

Table 11 Antimicrobial activity against derivatives *Pseudomonas aeruginosa* among the extracts from selected plants bearing quinonoids and standard quinone derivatives

Samples	Parts used	Solvent extracts	*Pseudomonas aeruginosa*		
			Inhibition zone (mm)	MIC (µg/ml)	MBC (µg/ml)
Plants					
Xyris indica	Flowers	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Cassia tora	Seeds	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Rhinacanthus nasutus	Roots	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
	Aerial parts	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Morinda elliptica	Roots	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Morinda citrifolia	Roots	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Morinda coreia	Roots	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Ardisia elliptica	Fruits	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Eleutherine americana	Bulbs	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Nigella sativa	Seeds	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Standard quinone derivatives					
Alizarin			NA	NA	NA
Juglone			NA	NA	NA
Lapachol			NA	NA	NA
Lawsone			NA	NA	NA
Embelin			NA	NA	NA
Positive controls					
Ampicllin sodium			NA	NA	NA
Amikacin sulfate			30.66±0.58	0.78	1.56
Negative control					
DMSO			NA	NA	NA

Means ± SD, NA = no activity, diameter of well = 6 mm. The tests were done in triplicate.

Table 12 Antimicrobial activity against derivatives *Salmonella typhi* among the extracts from selected plants bearing quinonoids and standard quinone derivatives

Samples	Parts used	Solvent extracts	*Salmonella typhi*		
			Inhibition zone (mm)	MIC (µg/ml)	MBC (µg/ml)
Plants					
Xyris indica	Flowers	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Cassia tora	Seeds	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Rhinacanthus nasutus	Roots	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
	Aerial parts	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Morinda elliptica	Roots	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Morinda citrifolia	Roots	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Morinda coreia	Roots	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Ardisia elliptica	Fruits	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Eleutherine americana	Bulbs	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Nigella sativa	Seeds	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Standard quinone derivatives					
Alizarin			NA	NA	NA
Juglone			NA	NA	NA
Lapachol			NA	NA	NA
Lawsone			12.67±0.58	>100	>100
Embelin			NA	NA	NA
Positive controls					
Ampicllin sodium			37.00±1.00	0.78	1.56
Amikacin sulfate			20.66±0.58	1.56	1.56
Negative control					
DMSO			NA	NA	NA

Means ± SD, NA = no activity, diameter of well = 6 mm. The tests were done in triplicate.

The ethanol extract from *Xyris indica* flowers, the petroleum ether extract from *Eleutherine americana* bulbs and juglone showed marginal potential against *Salmonella typhimurium* with the inhibition zone around 10-11 cm. The MIC and MBC of *Xyris indica* extract were more than 2,000 µg/ml. The MIC and MBC of *Eleutherine americana* extract were 500 and >2,000 µg/ml respectively. The MIC and MBC of juglone were 100 and >100 µg/ml respectively. The results were shown in Table 13.

Table 13 Antimicrobial activity against derivatives *Salmonella typhimurium* among the extracts from selected plants bearing quinonoids and standard quinone derivatives

Samples	Parts used	Solvent extracts	*Salmonella typhimurium*		
			Inhibition zone (mm)	MIC (µg/ml)	MBC (µg/ml)
Plants					
Xyris indica	Flowers	Petroleum ether	NA	NA	NA
		Ethanol	11.00±1.00	>2000	>2000
Cassia tora	Seeds	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Rhinacanthus nasutus	Roots	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
	Aerial parts	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Morinda elliptica	Roots	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Morinda citrifolia	Roots	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Morinda coreia	Roots	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Ardisia elliptica	Fruits	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Eleutherine americana	Bulbs	Petroleum ether	11.00±0.00	500	>2000
		Ethanol	NA	NA	NA
Nigella sativa	Seeds	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Standard quinone derivatives					
Alizarin			NA	NA	NA
Juglone			10.33±0.58	100	>100
Lapachol			NA	NA	NA
Lawsone			NA	NA	NA
Embelin			NA	NA	NA
Positive controls					
Ampicllin sodium			40.00±0.00	0.39	0.78
Amikacin sulfate			30.00±0.00	0.78	0.78
Negative control					
DMSO			NA	NA	NA

Means ± SD, NA = no activity, diameter of well = 6 mm. The tests were done in triplicate.

Eight extracts and two standard quinone derivatives were slightly active against *Shigella spp.* The petroleum ether extract from *Eleutherine americana* bulb and lawsone showed the large zone of 17.33±0.58 and 14.66±0.58 mm respectively. For the MIC and MBC value, the ethanol extract from *Morinda citrifolia* roots and the petroleum ether extract from *Eleutherine americana* bulbs showed the lowest concentration of MIC at 500 µg/ml while juglone and lawsone showed the MIC at 100 µg/ml and MBC >100 µg/ml. The results were shown in Table 14.

Table 14 Antimicrobial activity against derivatives *Shigella sp.* among the extracts from selected plants bearing quinonoids and standard quinone derivatives

Samples	Parts used	Solvent extracts	*Shigella sp.*		
			Inhibition zone (mm)	MIC (µg/ml)	MBC (µg/ml)
Plants					
Xyris indica	Flowers	Petroleum ether	NA	NA	NA
		Ethanol	12.66±0.58	>2000	>2000
Cassia tora	Seeds	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Rhinacanthus nasutus	Roots	Petroleum ether	NA	NA	NA
		Ethanol	12.00±0.00	>2000	>2000
	Aerial parts	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Morinda elliptica	Roots	Petroleum ether	NA	NA	NA
		Ethanol	12.33±0.58	>2000	>2000
Morinda citrifolia	Roots	Petroleum ether	NA	NA	NA
		Ethanol	10.33±0.58	500	>2000
Morinda coreia	Roots	Petroleum ether	NA	NA	NA
		Ethanol	10.33±0.58	1000	>2000
Ardisia elliptica	Fruits	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Eleutherine americana	Bulbs	Petroleum ether	17.33±0.58	500	>2000
		Ethanol	13.00±0.00	>2000	>2000
Nigella sativa	Seeds	Petroleum ether	NA	NA	NA
		Ethanol	7.33±0.58	>2000	>2000
Standard quinone derivatives					
Alizarin			NA	NA	NA
Juglone			14.00±0.00	100	>100
Lapachol			NA	NA	NA
Lawsone			14.66±0.58	100	>100
Embelin			NA	NA	NA
Positive controls					
Ampicllin sodium			33.00±0.00	3.12	6.25
Amikacin sulfate			41.00±1.00	0.78	1.56
Negative control					
DMSO			NA	NA	NA

Means ± SD, NA = no activity, diameter of well = 6 mm. The tests were done in triplicate.

The antifungal activities were performed against *Candida albicans* and *Saccharomyces cerevisiae*. Among 20 crude extracts and 5 standard quinone derivatives, they were found that 11 extracts and 3 standard quinone derivatives were marginally active against *Candida albicans*. Both extracts of *Morinda citrifolia* roots and the petroleum ether extract of *Morinda coreia* had more potency than other extracts with MIC of 125 and 250 µg/ml and MFC of 250 and 500 µg/ml respectively.

Alizarin, juglone and lawsone were 3 quinones capable to *Escherichia coli* inhibition. The results were shown in Table 15.

Table 15 Antimicrobial activity against derivatives *Candida albicans* among the extracts from selected plants bearing quinonoids and standard quinone derivatives

Samples	Parts used	Solvent extracts	*Candida albicans*		
			Inhibition zone (mm)	MIC (µg/ml)	MBC (µg/ml)
Plants					
Xyris indica	Flowers	Petroleum ether	NA	NA	NA
		Ethanol	9.67±0.58	2000	>2000
Cassia tora	Seeds	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Rhinacanthus nasutus	Roots	Petroleum ether	10.00±0.00	>2000	>2000
		Ethanol	NA	NA	NA
	Aerial parts	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Morinda elliptica	Roots	Petroleum ether	8.67±0.58	1000	>2000
		Ethanol	9.67±0.58	2000	>2000
Morinda citrifolia	Roots	Petroleum ether	9.67±0.58	125	250
		Ethanol	13.00±00	250	500
Morinda coreia	Roots	Petroleum ether	12.67±0.58	250	500
		Ethanol	9.33±0.58	2000	>2000
Ardisia elliptica	Fruits	Petroleum ether	NA	NA	NA
		Ethanol	7.67±0.58	250	2000
Eleutherine americana	Bulbs	Petroleum ether	14.00±0.00	250	1000
		Ethanol	10.00±0.00	2000	>2000
Nigella sativa	Seeds	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Standard quinone derivatives					
Alizarin			9.67±0.658	100	50
Juglone			28.00±2.00	100	>100
Lapachol			NA	NA	NA
Lawsone			10.67±2.10	100	>100
Embelin			NA	NA	NA
Positive controls					
Ampicllin sodium			NA	NA	NA
Amikacin sulfate			NA	NA	NA
Negative control					
DMSO			NA	NA	NA

Means ± SD, NA = no activity, diameter of well = 6 mm. The tests were done in triplicate.

Both extracts from *Cassia tora* seeds and *Nigella sativa* seeds as well as the petroleum ether extract from *Ardisia elliptica* fruits showed no inhibitory activity against *Saccharomyces cerevisiae*. The other plant extracts exhibited slightly inhibitory activity according to MIC of more than 250 µg/ml and MFC of more than

2,000 μg/ml. Lawsone was the only quinones possessed inhibitory activity against *Saccharomyces cerevisiae* with the MIC of 25 μg/ml and MFC of more than 100 μg/ml. The results were shown in Table 16.

Table 16 Antimicrobial activity against derivatives *Saccharomyces cerevisiae* among the extracts from selected plants bearing quinonoids and standard quinone derivatives

Samples	Parts used	Solvent extracts	*Saccharomyces cerevisiae*		
			Inhibition zone (mm)	MIC (μg/ml)	MBC (μg/ml)
Plants					
Xyris indica	Flowers	Petroleum ether	13.67±0.58	500	>2000
		Ethanol	11.00±0.00	250	>2000
Cassia tora	Seeds	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Rhinacanthus nasutus	Roots	Petroleum ether	20.00±0.00	>2000	>2000
		Ethanol	15.33±0.58	>2000	>2000
	Aerial parts	Petroleum ether	11.00±0.00	>2000	>2000
		Ethanol	16.67±0.58	>2000	>2000
Morinda elliptica	Roots	Petroleum ether	10.00±0.00	500	>2000
		Ethanol	13.00±0.00	500	>2000
Morinda citrifolia	Roots	Petroleum ether	12.00±0.00	250	1000
		Ethanol	15.00±0.00	500	2000
Morinda coreia	Roots	Petroleum ether	10.33±0.58	500	>2000
		Ethanol	16.33±0.58	2000	>2000
Ardisia elliptica	Fruits	Petroleum ether	NA	NA	NA
		Ethanol	14.33±0.58	2000	>2000
Eleutherine americana	Bulbs	Petroleum ether	14.00±0.00	250	>2000
		Ethanol	17.67±0.58	250	>2000
Nigella sativa	Seeds	Petroleum ether	NA	NA	NA
		Ethanol	NA	NA	NA
Standard quinone derivatives					
Alizarin			NA	NA	NA
Juglone			NA	NA	NA
Lapachol			NA	NA	NA
Lawsone			10.00±0.00	25	>100
Embelin			NA	NA	NA
Positive controls					
Ampicllin sodium			NA	NA	NA
Amikacin sulfate			NA	NA	NA
Negative control					
DMSO			NA	NA	NA

Means ± SD, NA = no activity, diameter of well = 6 mm. The tests were done in triplicate.

CHAPTER V

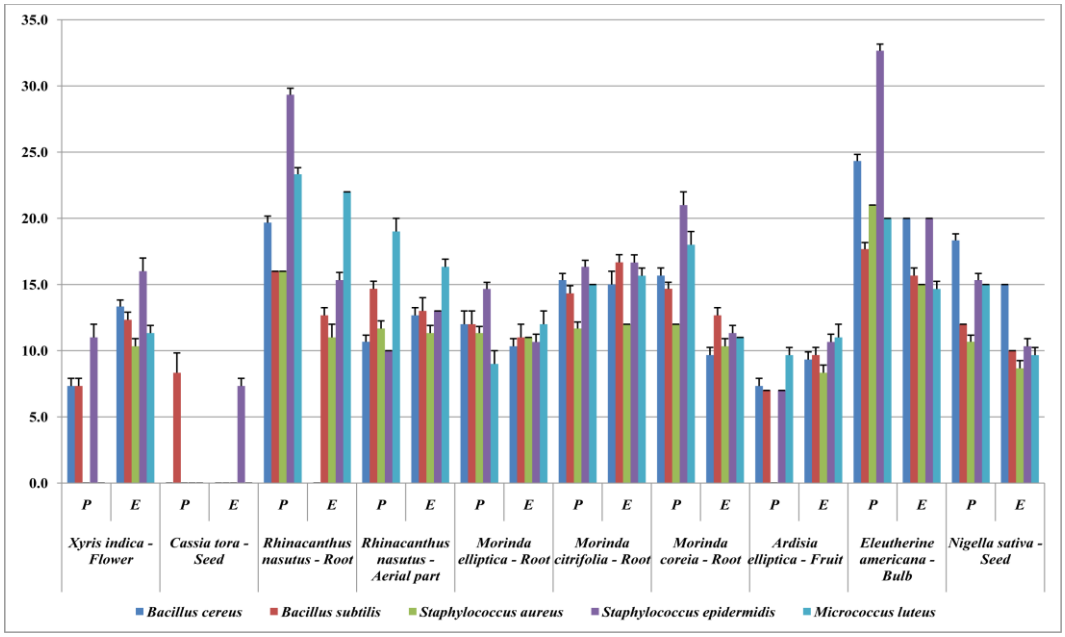
DISCUSSION AND CONCLUSION

Plants are the important sources for the development of new therapeutic drugs. In recent years, many phytochemical or bioactive compounds are derived from plants. Many researchers have spent over a hundred years trying to screen new antibiotic drug candidates from herbal medicines. Antimicrobial susceptibility testing is the first step towards for use to search new antibiotic, it could be predict the *in vivo* success or failure of antibiotic therapy.

The petroleum ether and ethanol extract from selected Thai medicinal plants bearing quinonoids including anthraquinones: *Xyris indica*, *Cassia tora*, *Morinda elliptica*, *Morinda citrifolia* and *Morinda coreia*; benzoquinones: *Ardisia elliptica* and *Nigella sativa*; and naphthoquinones: *Rhinacanthus nasutus* and *Eleutherine americana* were investigated for their antimicrobial activities. The quinone derivative compounds such as alizarin, juglone, lapachol, lawsone and embelin were used as a reference compounds.

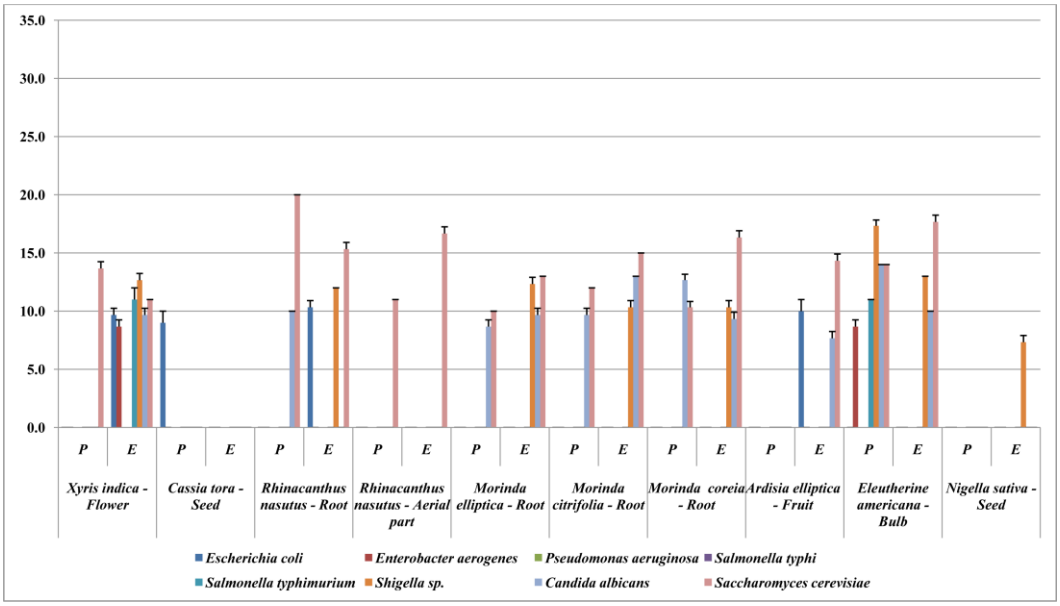
The results demonstrated that the petroleum ether extract from *Xyris indica* flowers showed the inhibitory effect against three gram positive bacteria and one fungi but there were no effect against gram negative bacteria. The ethanol extract from this plant exhibited the antimicrobial activity toward five gram positive bacteria, four gram negative bacteria and two fungi (Figure 18-20 and Table 17-19). It also displayed a large inhibition zone of 16.00±1.00 mm on *Staphylococcus epidermidis* while the petroleum ether extract showed the lowest MIC on *Staphylococcus epidermidis* and the lowest MBC on *Bacillus subtilis* (62.50 and 1000 µg/ml respectively). The previous study was found that the extract from *Xyris indica* flowers exhibited antibacterial activity on gram positive bacteria and showed good antifungal activity on dermatophytes as well as ringworm [35]. Active chemical compounds were proposed to be anthraquinone derivatives such as chysazin, 3-methoxychrysazin and 3-hydroxy-chrysazinchrysazin. It was reported that

these substances were isolated from flower of *Xyris indica* [35,37] and were found in *Xyris* spices [142].



P = Petroleum ether extracts , *E* = Ethanol extracts

Figure 18 Spectrum of antimicrobial activity against gram positive bacteria



P = Petroleum ether extracts , *E* = Ethanol extracts

Figure 19 Spectrum of antimicrobial activity against gram negative bacteria and fungi

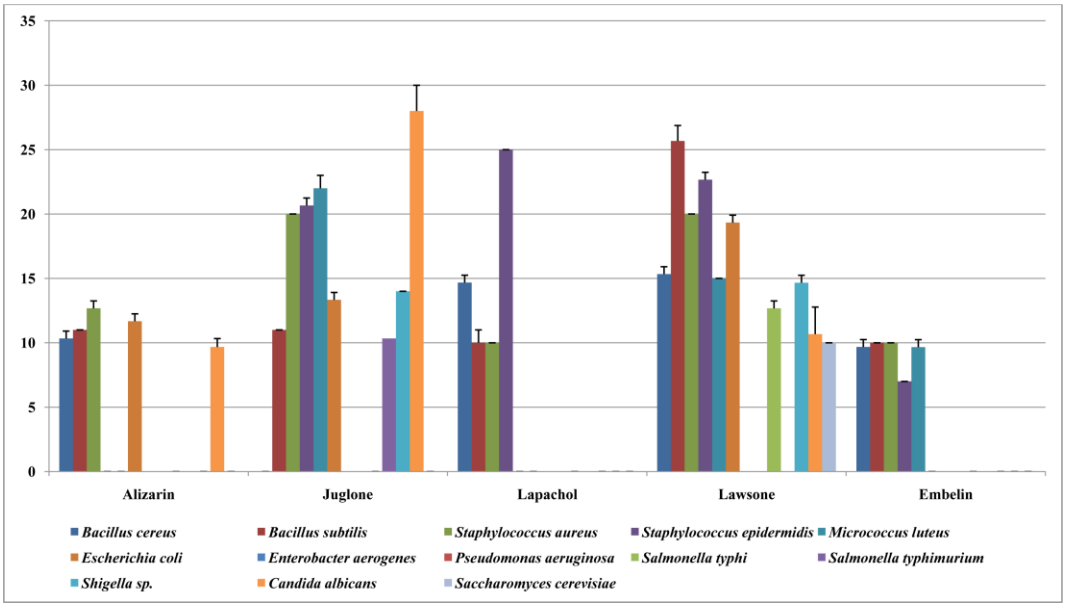


Figure 20 Spectrum of antimicrobial activity from standard quinone derivatives

The petroleum ether extract from seeds of *Cassia tora* showed the potential against only gram positive bacteria, *Bacillus subtilis* and gram negative bacteria, *Escherichia coli*. No activity was observed against fungi. The ethanol extract inhibited only gram positive bacteria, *Staphylococcus epidermidis* with the MIC of 2000 µg/ml. However, the previous studies found the different results on *Cassia tora*. That the petroleum ether extract of *Cassia tora* did not show the potential on *Bacillus subtilis* and *Escherichia coli* whereas the ethanolic extract of this plant possessed the inhibitory effect against *Bacillus subtilis* [46]. In addition, Menghani and Soni (2012) reported antimicrobial activity of petroleum ether extract from *Cassia tora* that the extract was active on *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* by disc diffusion method at 8 mg/ disc concentration [143]. This study did not showed similar activity against those four microbes. It might be due to different method and concentration. This study used well diffusion method at 4 mg/well concentration. The expected active chemicals were anthraquinone derivatives found in this plant such as chrysophanol, physcion, emodin, rhein, euphol, basseol, obtusifolin,

obtusin, chryso-obtusin, rubrofusarin, aurantio-obtusin, chrysophonic acid-9-anthrone [41-44].

Both petroleum ether and ethanolic extract from *Morinda elliptica*, *Morinda citrifolia* and *Morinda coreia* roots showed the similar potency against the tested microorganisms. The petroleum ether and ethanol extracts exhibited the inhibitory activities against five gram positive bacteria and two fungi while the petroleum ether extract was not effect against gram negative bacteria. The ethanol extract demonstrated the antimicrobial activity against only gram negative bacteria, *Shigella sp*. The petroleum ether extract from *Morinda coreia* showed large inhibition zone of 21.00±1.00 mm on *Staphylococcus epidermidis* and the petroleum ether extract from *Morinda citrifolia* showed the lowest MIC and MBC on *Micrococcus luteus* and *Candida albicans* at 125 and 250 µg/ml respectively. This study found similar result for *Morinda elliptica* as previously reported that anthraquinones isolated from the roots of this plant showed potent antimicrobial activity against *Pseudomonas aeruginosa*, *Aspergillus ochraceus, Aspergillus niger* and *Candida lypolitica*. 2-Formyl-1-hydroxyanthraquinone and damnacanthal were active compounds against *Pseudomonas aeruginosa*. For antifungal activity, damnacanthal and nordamnacanthal showed very strong activity against all the tested fungi whereas morindone showed very strong antifungal activity against only *Candida lipolytica* [51]. For previous study of *Morinda citrifolia*, it was found that the compounds from roots showed good activity against both gram positive and gram negative bacterial strains at the high concentrations such as *Pseudomonas aeruginosa, Proteus morgaii, Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Salmonella sp.* and *Shigella sp.*[71,72]. In addition, the extracts from the riped *Morinda citrifolia* fruits was reported antibacterial activities against *Pseudomonas aeruginosa*, *Micrococcus pyrogenes*, *Escherichia coli*, *Salmonella typhosa*, *Salmonella montevideo*, *Salmonella schottmuelleri* and *Shigella paradys* [70] and the juice extract from fruits was reported antifungal effect on *Candida albicans* [73]. Furthermore, the activities of the methanolic and aqueous extracts from the leaves of *Morinda coreia* were displayed on gram positive and gram negative bacteria. The methanolic extract was more active on gram positive

bacteria (*Staphylococcus aureus*) and least active on gram negative bacteria (*Escherichia coli*). The aqueous extract was more active on gram positive bacteria (*Bacillus subtilis*) and least active on gram negative bacteria (*Escherichia coli*) [79]. Anthraquinones derivatives were major phytochemical constituents obtained mainly from the roots and fruits of the *Morinda* genus. These compounds were proposed for antimicrobial activity of *Morinda* genus [74].

The petroleum ether extract from the fruits of *Ardisia elliptica* exhibited the inhibitory effect toward four gram positive bacteria except *Staphylococcus aureus*, but there was no effect on fungi and gram negative bacteria. The ethanol extract inhibited five gram positive bacteria and also *Escherichia coli* as well as *Saccharomyces cerevisiae*. The ethanol extract showed large inhibition zone of 14.33±0.58 mm on *Saccharomyces cerevisiae.* The lowest MIC and MBC were from petroleum ether extract against *Bacillus subtilis* at 62.50 µg/ml. The results were in discordance with previous reports that the ethanol extract from fruits showed the activity against gram negative bacteria, both *Salmonella spp.* and *Shigella spp.* [95,144]. The compound possessed antimicrobial activity was embelin. This benzoquinone derivative is a major component of phytochemical constituents obtained in Myrsinaceae family, especially in *Ardisia sp.* and *Embelia sp*. [89,145]. This study reported the antimicrobial properties of *Ardisia elliptica* fruits against gram positive, gram negative bacteria and fungi.

The petroleum ether and ethanol extracts from seeds of *Nigella sativa* showed the inhibitory activity against five gram positive bacteria. For gram negative bacteria and fungi, they were not active except that the ethanol extract inhibited only *Shigella spp.* The petroleum ether extract showed large inhibition zone of 18.33±0.58 mm on *Basillus cereus* and showed the lowest MIC and MBC at 125 µg/ml on *Bacillus subtilis*. Salman *et al.* reported that the activity from oil of seeds showed more inhibition against gram positive than gram negative bacteria [109]. However, Zuridah *et al*. reported that the methanolic extracts from seeds were active on *Staphylococcus aureus* and *Pseudomonas aeruginosa* growth inhibition, but inactive on *Escherichia coli* and *Bacillus cereus* [108].

The fixed oil also exhibited the good antifungal activity against *Candida parapsilosis* and *Candida glabata* [110]. The antimicrobial activity of this *Nigella sativa* oil might be attributed to the presence of benzoquinone derivatives, thymoquinone and thymohydroquinone in the oil. Both compounds were demonstrated antimicrobial properties [109, 146-149].

The petroleum ether extract from the roots of *Rhinacanthus nasutus* exhibited antimicrobial activity against five gram positive bacteria, two fungi and gram negative bacteria. The ethanol extract showed the activity against five gram positive bacteria. Two gram negative bacteria, *Escherichia coli* and *Shigella spp.* as well as fungi, *Saccharomyces cerevisiae* were also inhibited. In addition, both extracts from aerial parts inhibited the growth of five gram positive bacteria, but inhibited gram negative bacteria only *Escherichia coli* and fungi only *Saccharomyces cerevisiae*. The petroleum ether extract from roots showed large inhibition zone of 29.33±0.58 mm and the lowest MIC and MBC on *Micrococcus luteus* at 3.90 and 15.62 µg/ml respectively. The previous studies revealed that the leaf extract from *Rhinacanthus nasutus* exhibited antimicrobial activity against gram positive aerobic bacteria, *Staphylococcus aureus* and *Staphylococcus epidermidis* with the MBC values of 256 and 512 µg/ml respectively. In addition, the extract exhibited stronger bactericidal activity against gram positive anaerobic but not active against *Candida albicans* even at concentration >2000 µg/ml. The activity of extract was almost equal to that of rhinacanthin-C. This might be due to high content of rhinacanthin derivatives in the extract and their synergistic effects [118] The significant bioactive ingredients of this plant were known to be naphthoquinone derivatives such as rhinacanthin-C, rhinacanthin-D, rhinacanthin-N, rhinacanthin-Q, rhinacanthins (A-D, G-Q), rhinacanthone [113,114].

Both extracts from blubs of *Eleutherine americana* inhibited the growth of five gram positive bacteria and two fungi. The petroleum ether extract presented potency against three gram negative bacteria, *Enterobacter aerogenes, Salmonella typhimurium* and *Shigella spp.* The ethanol extract showed the activity against only *Shigella spp*. For

the inhibition zone, the petroleum ether extract showed large zone of 32.66±0.58 mm on *Staphylococcus epidermidis* and presented the lowest MIC and MBC values of 62.50 and 125 µg/ml respectively on *Basillus cereus*. Beatrice *et al.* (2010) reported that the crude ethanolic extract from bulbs of *Eleutherine americana* showed good antibacterial activities against all gram positive bacteria tested but only gram negative bacteria tested, *Erwinia* spp was active. Furthermore, the extract exhibited antifungal activity against *Aspergillus niger, Rhizopus* spp. and *Penicillium* spp. The dermatophyte fungi and yeasts were resistant to the extract. In addition, the result from that study revealed that the extract displayed more inhibitory activity against gram positive than gram negative bacteria [130]. The high content of bioactive ingredient of this plant were known to be naphthoquinones, naphthalene and their derivatives such as hongconin, elecanacin, eleuthoside B, isoeleutherin, eleutherin, eleutherol eleutherinoside A [123-126].

Among five species from selected Thai medicinal plants that bearing anthraquinones, the petroleum ether extract from *Morinda citrifolia* roots showed highest potency against gram positives bacteria with MIC and MBC of 125 µg/ml on *Bacillus subtilis* and showed activity against fungi with the lowest MIC and MBC on *Candida albicans* at 125 and 250 µg/ml respectively. Similar to the extracts, standard quinone derivatives, alizarin showed highest activity against gram positive bacteria with the lowest MIC of 12.50 and MBC of >100 µg/ml on *Staphylococcus aureus* and the lowest MIC and MFC values of 100 and 50 µg/ml respectively against *Candida albicans*.

For Thai medicinal plants bearing benzoquinones, it was found that the petroleum ether extract from *Ardisia elliptica* fruits presented good activity against gram positive bacteria *Bacillus subtilis* with the lowest MIC and MBC of 62.50 µg/ml. Likewise standard quinone derivatives, embelin exhibited activity against five gram positive bacteria with the lowest MIC and MBC at 6.25 µg/ml on *Bacillus subtilis* and *Bacillus cereus*. In addition, Thai medicinal plants bearing naphthoquinones were found that the petroleum ether extract from *Rhinacanthus nasutus* roots possessed highest potency with the lowest MIC and MBC of 3.90 and 15.62 µg/ml respectively against gram positives

Micrococcus luteus. For three standard naphthoquinone derivatives, each compound presented good antimicrobial activity against gram positive bacteria. Lawsone showed highest activity on *B. cereus* with the lowest MIC and MBC at 25 and 100 µg/ml respectively.

Cassia tora possessed least spectrum of antimicrobial activity as well as least potency according to inhibition zone diameters, MIC and MBC. *Eleutherine americana* especially petroleum ether extract and *Xyris indicae* specially ethanol extract expressed broadest spectrum of antimicrobial activity. Three species of *Morinda* showed similar spectrum and potency against tested microorganisms.

The mechanism of action on antimicrobial potential of quinonoids was associated with electron reduction of quinone to hydroquinone moiety. The hydroxyl groups could covalently bonded with DNA to form DNA adduct resulting in mitotic blockage. The redox reaction of quinone compounds could also generate reactive oxygen species especially hydroxyl radicals which responsible to irreversibly complex formation with lipids and proteins in the microbial cell. These reactions might interfere to surface-exposed adhesins, cell-wall polypeptides, and membrane-bound enzymes of the microorganisms. [150-152].

The potential of quinone antimicrobial effects is evidential. However, there are a range of antimicrobial efficacies.The investigation of plants bearing quinonoids as well as standard quinone derivatives in this study found that naphthoquinones (*Eleutherine americana* extract, *Rhinacanthus nasutus* extract, lawsone and juglone) showed highest range and potency in antimicrobial activity. However, lapachol, one of naphthoquinone derivatives showed lesser potent action. This might be due to the substituent functional groups presented on the benzene ring. Lawsone and juglone are chemically similar, while lapachol has the side chain of $CH=C(OH)CH(CH3)_2$ (Figure 7). The difference in chemical structure affects both affinity of compound to target site and alsocompound solubility [150]. Herbal medicine has more complicated mechanism of action because of

a variety of compounds. Synergistic or antagonistic interaction plays an important role in efficacy of treatment.

The present study revealed the antimicrobial potentials among selected Thai medicinal plants bearing quinonoid compounds. The results could expand our knowledge in Thai traditional plant usages and discloses Thai traditional wisdom. Furthermore, the antimicrobial activities against pathogenic as well as antibiotic resistant microorganisms were recommended.

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APPENDIX

Table 17 Inhibition zone of gram positive bacteria

Samples	Parts used	Solvent extracts	Inhibition zone of gram positive bacteria (mm.)				
			Bacillus cereus	*Bacillus subtilis*	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Micrococcus luteus*
Plants							
Xyris indica	Flower	Pet. ether	7.33±0.58	7.33±0.58	NA	11.00±1.00	NA
		Ethanol	13.33±0.58	12.33±0.58	10.33±0.58	16.00±1.00	11.33±0.58
Cassia tora	Seed	Pet. ether	NA	8.33±1.50	NA	NA	NA
		Ethanol	NA	NA	NA	7.33±0.58	NA
Rhinacanthus nasutus	Root	Pet. ether	19.67±0.58	16.00±0.00	16.00±0.00	29.33±0.58	23.33±0.58
		Ethanol	NA	12.67±0.58	11.00±1.00	15.33±0.58	22.00±0.00
	Aerial part	Pet. ether	10.67±0.58	14.67±0.58	11.67±0.58	10.00±0.00	19.00±1.00
		Ethanol	12..67±0.58	13.00±1.00	11.33±0.58	13.00±0.00	16.33±0.58
Morinda elliptica	Root	Pet. ether	12.00±1.00	12.00±1.00	11.33±0.58	14.66±0.58	9.00±1.00
		Ethanol	10.33±0.58	11.00±1.00	11.00±0.00	10.66±0.58	12.00±1.00
Morinda citrifolia	Root	Pet. ether	15.33±0.58	14.33±0.58	11.67±0.58	16.33±0.58	15.00±0.00
		Ethanol	15.00±1.00	16.67±0.58	12.00±0.00	16.66±0.58	15.66±0.58
Morinda coreia	Root	Pet. ether	15. 67±0.58	14.67±0.58	12.00±0.00	21.00±1.00	18.00±1.00
		Ethanol	9.67±0.58	12.67±0.58	10.33±0.58	11.33±0.58	11.00±0.00
Ardisia elliptica	Fruit	Pet. ether	7.33±0.58	7.00±0.00	NA	7.00±0.00	9.66±0.58
		Ethanol	9.33±0.58	9.67±0.58	8.33±0.58	10.66±0.58	11.00±1.00
Eleutherine americana	Bulb	Pet. ether	24.33±0.58	17.67±0.58	21.00±0.00	32.66±0.58	20.00±0.00
		Ethanol	20.00±0.00	15.67±0.58	15.00±0.00	20.00±0.00	14.66±0.58
Nigella sativa	Seed	Pet. ether	18.33±0.58	12.00±0.00	10.67±0.58	15.33±0.58	15.00±0.00
		Ethanol	15.00±0.00	10.00±0.00	8.67±0.58	10.33±0.58	9.66±0.58
Standard quinone derivatives							
Alizarin			10.33±0.58	11.00±0.00	12.67±0. 0.58	NA	NA
Juglone			NA	11.00±0.00	20.00±0.00	20.66±0.58	22.00±1.00
Lapachol			14. 67±0.58	10.00±1.00	10.00±0.00	25.00±0.00	NA
Lawsone			15.33±0.58	25.67±1.20	20.00±0.00	22.66±0.58	15.00±0.00
Embelin			9. 67±0.58	10.00±0.00	10.00±0.00	7.00±0.00	9.66±0.58
Positive controls							
Ampicllin sodium			40.33±0.58	19.33±0.58	50.33±0.58	30.66±0.58	46.00±1.00
Amikacin sulfate			32.00±0.00	29.33±0.58	30.00±0.00	31.33±0.58	30.00±0.00
Negative control							
DMSO			NA	NA	NA	NA	NA

Means ± SD, NA = no activity, diameter of well = 6 mm. The tests were done in triplicate.

Table 18 Inhibition zone of gram negative bacteria

Samples	Parts used	Solvent extracts	Inhibition zone of gram negative bacteria (mm.)					
			Escherichia coli	***Enterobacter aerogenes***	***Pseudomonas aeruginosa***	***Salmonella typhi***	***Salmonella typhimurium***	***Shigella sp.***
Plants								
Xyris indica	Flower	Pet. ether	NA	NA	NA	NA	NA	NA
		Ethanol	9.67±0.58	8.67±0.58	NA	NA	11.00±1.00	12.66±0.58
Cassia tora	Seed	Pet. ether	9.00±1.00	NA	NA	NA	NA	NA
		Ethanol	NA	NA	NA	NA	NA	NA
Rhinacanthus nasutus	Root	Pet. ether	NA	NA	NA	NA	NA	NA
		Ethanol	10.33±0.58	NA	NA	NA	NA	12.00±0.00
	Aerial part	Pet. ether	NA	NA	NA	NA	NA	NA
		Ethanol	NA	NA	NA	NA	NA	NA
Morinda elliptica	Root	Pet. ether	NA	NA	NA	NA	NA	NA
		Ethanol	NA	NA	NA	NA	NA	12.33±0.58
Morinda citrifolia	Root	Pet. ether	NA	NA	NA	NA	NA	NA
		Ethanol	NA	NA	NA	NA	NA	10.33±0.58
Morinda coreia	Root	Pet. ether	NA	NA	NA	NA	NA	NA
		Ethanol	NA	NA	NA	NA	NA	10.33±0.58
Ardisia elliptica	Fruit	Pet. ether	NA	NA	NA	NA	NA	NA
		Ethanol	10.00±1.00	NA	NA	NA	NA	NA
Eleutherine americana	Bulb	Pet. ether	NA	8.67±0.58	NA	NA	11.00±0.00	17.33±0.58
		Ethanol	NA	NA	NA	NA	NA	13.00±0.00
Nigella sativa	Seed	Pet. ether	NA	NA	NA	NA	NA	NA
		Ethanol	NA	NA	NA	NA	NA	7.33±0.58
Standard quinone derivatives								
Alizarin			11.67±0.58	NA	NA	NA	NA	NA
Juglone			13.33±0.58	NA	NA	NA	10.33±0.58	14.00±0.00
Lapachol			NA	NA	NA	NA	NA	NA
Lawsone			19.33±0.58	NA	NA	12.67±0.58	NA	14.66±0.58
Embelin			NA	NA	NA	NA	NA	NA
Positive controls								
Ampicllin sodium			32.00±0.00	18.66±0.58	NA	37.00±1.00	40.00±0.00	33.00±0.00
Amikacin sulfate			21.00±0.00	23.33±0.58	30.66±0.58	20.66±0.58	30.00±0.00	41.00±1.00
Negative control								
DMSO			NA	NA	NA	NA	NA	NA

Means ± SD, NA = no activity, diameter of well = 6 mm. The tests were done in triplicate.

Table 19 Inhibition zone of fungi

Samples	Parts used	Solvent extracts	Inhibition zone of fungi (mm.)	
			Candida albicans	*Saccharomyces cerevisiae*
Plants				
Xyris indica	Flower	Petroleum ether	NA	13.67±0.58
		Ethanol	9.67±0.58	11.00±0.00
Cassia tora	Seed	Petroleum ether	NA	NA
		Ethanol	NA	NA
Rhinacanthus nasutus	Root	Petroleum ether	10.00±0.00	20.00±0.00
		Ethanol	NA	15.33±0.58
	Aerial part	Petroleum ether	NA	11.00±0.00
		Ethanol	NA	16.67±0.58
Morinda elliptica	Root	Petroleum ether	8.67±0.58	10.00±0.00
		Ethanol	9.67±0.58	13.00±0.00
Morinda citrifolia	Root	Petroleum ether	9.67±0.58	12.00±0.00
		Ethanol	13.00±00	15.00±0.00
Morinda coreia	Root	Petroleum ether	12.67±0.58	10.33±0.58
		Ethanol	9.33±0.58	16.33±0.58
Ardisia elliptica	Fruit	Petroleum ether	NA	NA
		Ethanol	7.67±0.58	14.33±0.58
Eleutherine americana	Bulb	Petroleum ether	14.00±0.00	14.00±0.00
		Ethanol	10.00±0.00	17.67±0.58
Nigella sativa	Seed	Petroleum ether	NA	NA
		Ethanol	NA	NA
Standard quinone derivatives				
Alizarin			9.67±0.658	NA
Juglone			28.00±2.00	NA
Lapachol			NA	NA
Lawsone			10.67±2.10	10.00±0.00
Embelin			NA	NA
Positive controls				
Ampicllin sodium			NA	NA
Amikacin sulfate			NA	NA
Negative control				
DMSO			NA	NA

Means ± SD, NA = no activity, diameter of well = 6 mm. The tests were done in triplicate.

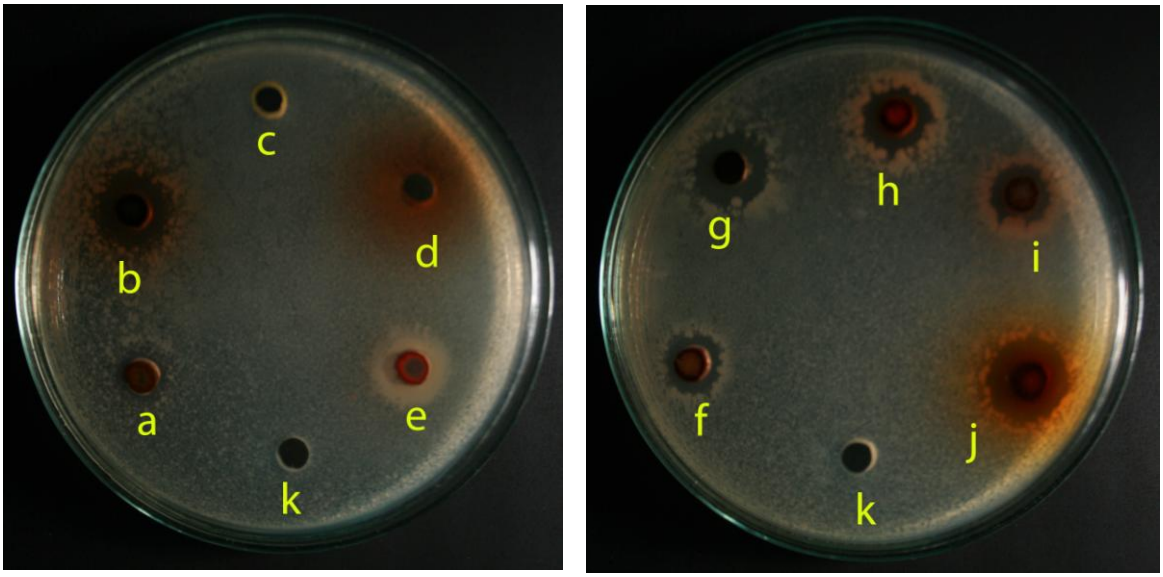

Figure 21 The inhibition zone of *Bacillus cereus* from *Xyris indica* L. (flower): **a.** petroleum ether extract, **b.** ethanol extract; *Cassia tora* Linn. (seed): **c.** petroleum ether extract, **d.** ethanol extract; *Rhinacanthus nasutus* (L.) Kurz (root): **e.** ethanol extract; *Rhinacanthus nasutus* (L.) Kurz (aerial part): **f.** petroleum ether extract, **g.** ethanol extract; *Morinda elliptica* Ridl. (root): **h.** petroleum ether extract, **i.** ethanol extract; *Morinda citrifolia* L. (root); **j.** petroleum ether extract; **k.** DMSO

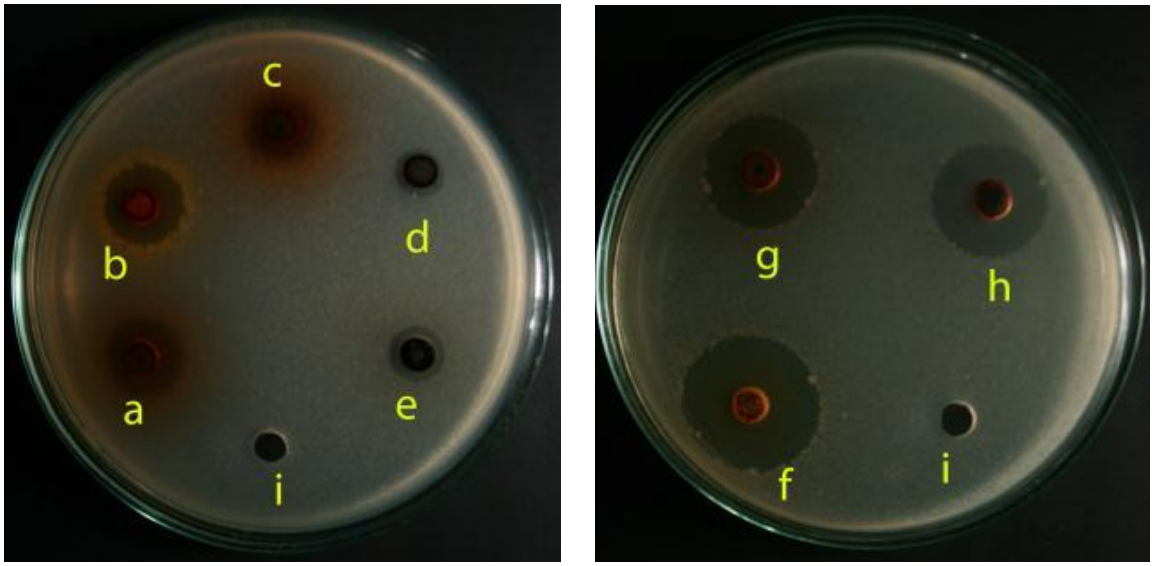

Figure 21 The inhibition zone of *Bacillus cereus* (cont.) from *Morinda citrifolia* L. (root): **a.** ethanol extract; *Morinda coreia* Ham. (root): **b.** petroleum ether extract, **c.** ethanol extract; *Ardisia elliptica* Thunb. (fruit): **d.** petroleum ether extract, **e.** ethanol extract; *Eleutherine Americana* (Aubl.) (bulb): **f.** petroleum ether extract, **g.** ethanol extract; *Rhinacanthus nasutus* (L.) Kurz (root): **h.** petroleum ether extract; **i.** DMSO

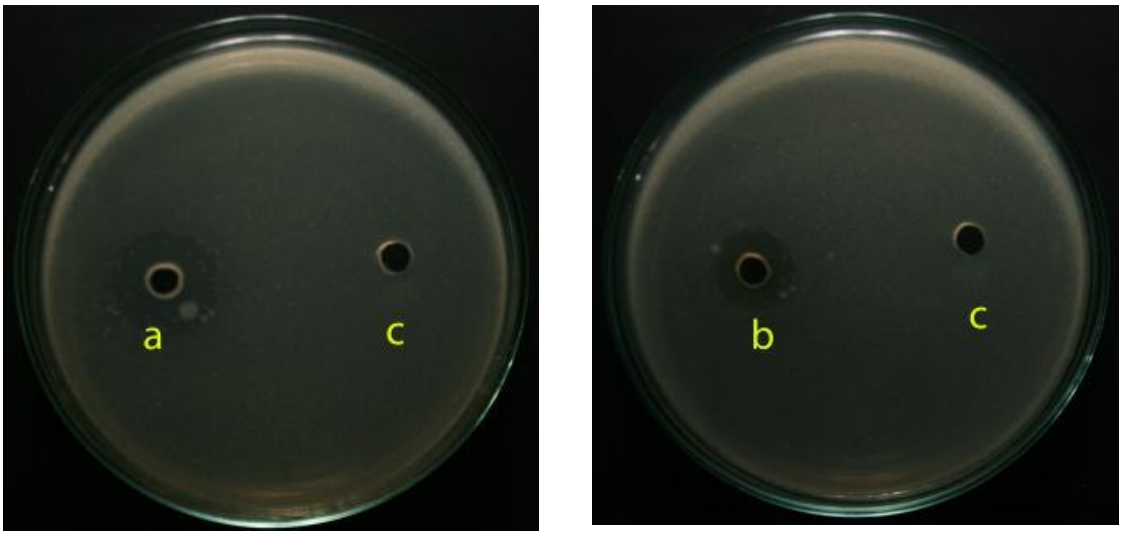

Figure 21 The inhibition zone of *Bacillus cereus* (cont.) from *Nigella sativa* Linn. (seed): **a.** petroleum ether extract, **b.** ethanol extract; **c.** DMSO

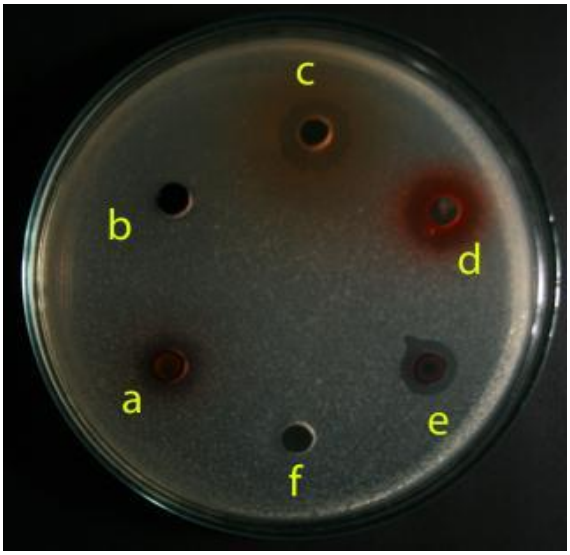

Figure 21 The inhibition zone of *Bacillus cereus* (cont.) from **a.** Alizarin; **b.** Juglone; **c.** Lawsone; **d.** Lapachol; **e.** Embelin; **f.** DMSO

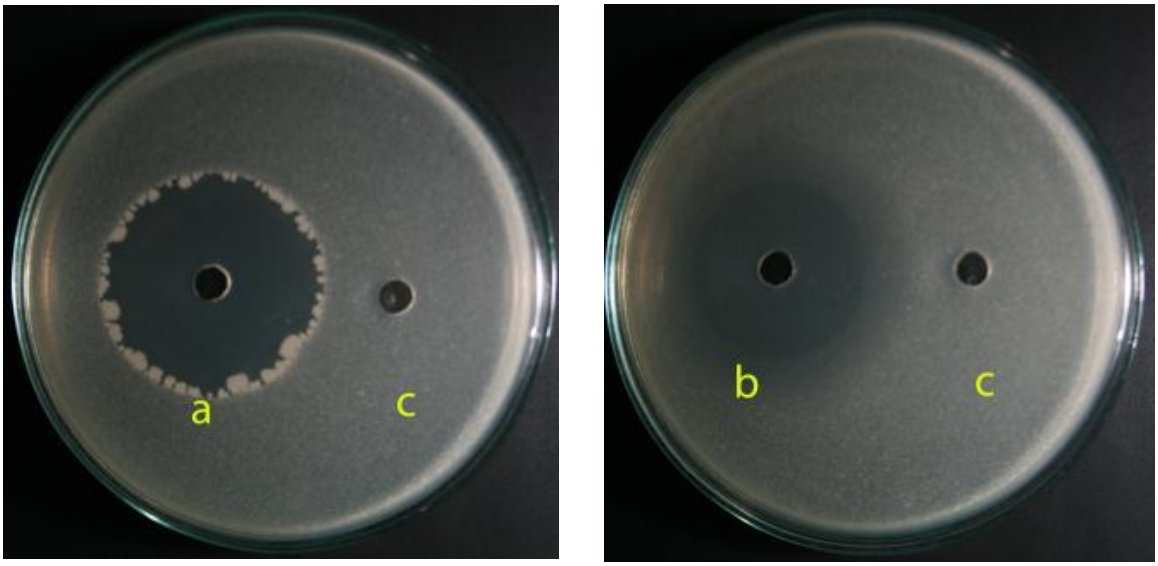

Figure 21 The inhibition zone of *Bacillus cereus* (cont.) from **a.** Ampicllin sodium; **b.** Amikacin sulfate; **c.** DMSO

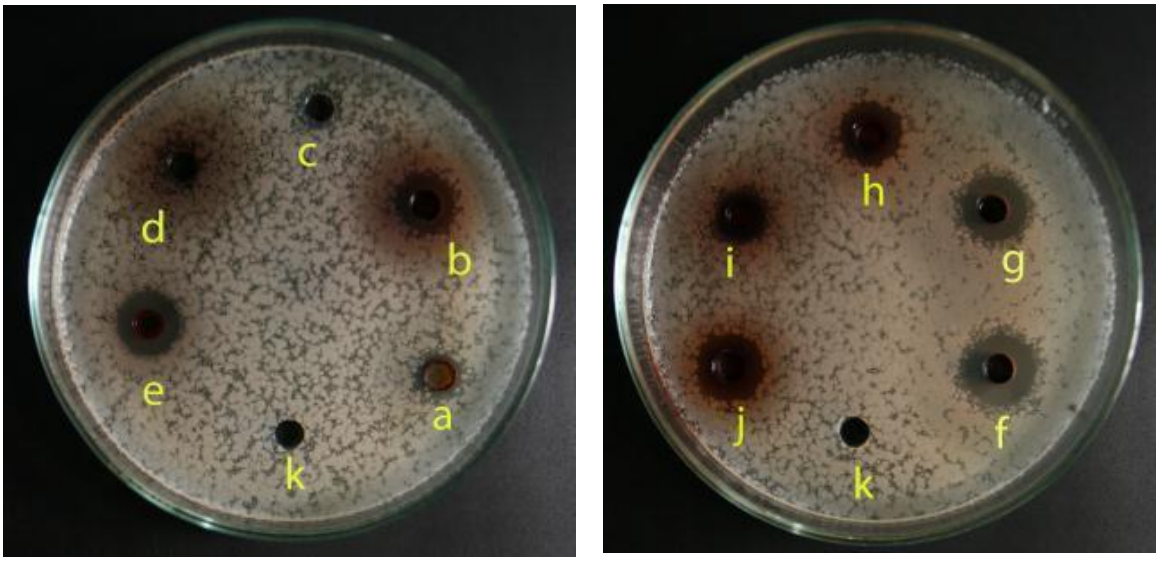

Figure 22 The inhibition zone of *Bacillus subtilis* from *Xyris indica* L. (flower): **a.** petroleum ether extract, **b.** ethanol extract; *Cassia tora* Linn. (seed): **c.** petroleum ether extract, **d.** ethanol extract; *Rhinacanthus nasutus* (L.) Kurz (root): **e.** ethanol extract; *Rhinacanthus nasutus* (L.) Kurz (aerial part): **f.** petroleum ether extract, **g.** ethanol extract; *Morinda elliptica* Ridl. (root): **h.** petroleum ether extract, **i.** ethanol extract; *Morinda citrifolia* L. (root): **j.** petroleum ether extract; **k.** DMSO

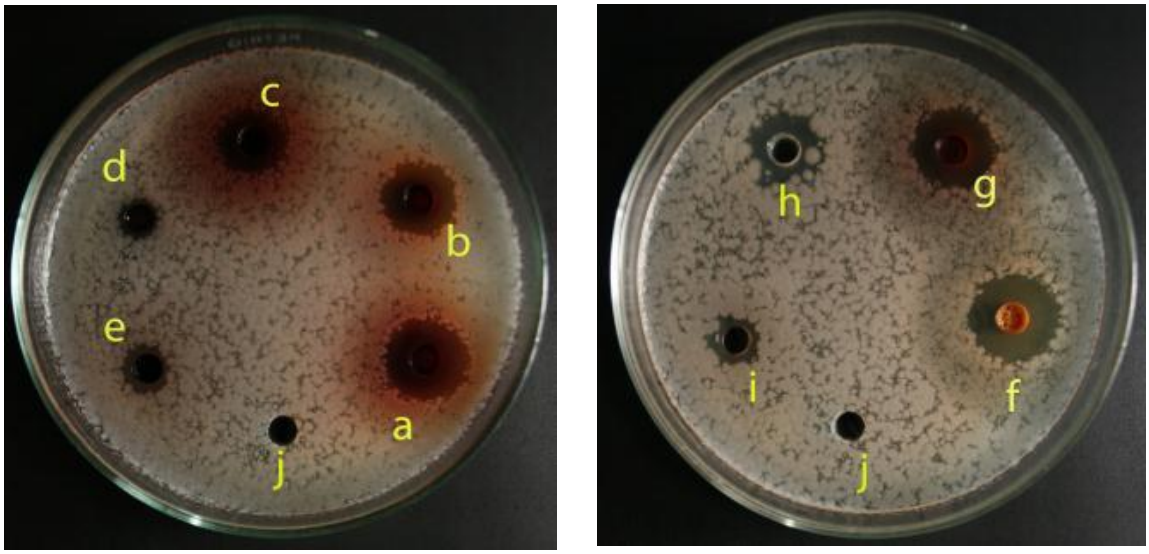

Figure 22 The inhibition zone of *Bacillus subtilis* (cont.) from *Morinda citrifolia* L. (root): **a.** ethanol extract; *Morinda coreia* Ham. (root): **b.** petroleum ether extract, **c.** ethanol extract; *Ardisia elliptica* Thunb. (fruit): **d.** petroleum ether extract, **e.** ethanol extract; *Eleutherine Americana* (Aubl.) (bulb): **f.** petroleum ether extract, **g.** ethanol extract; *Nigella sativa* Linn. (seed): **h.** petroleum ether extract, **i.** ethanol extract; **j.** DMSO

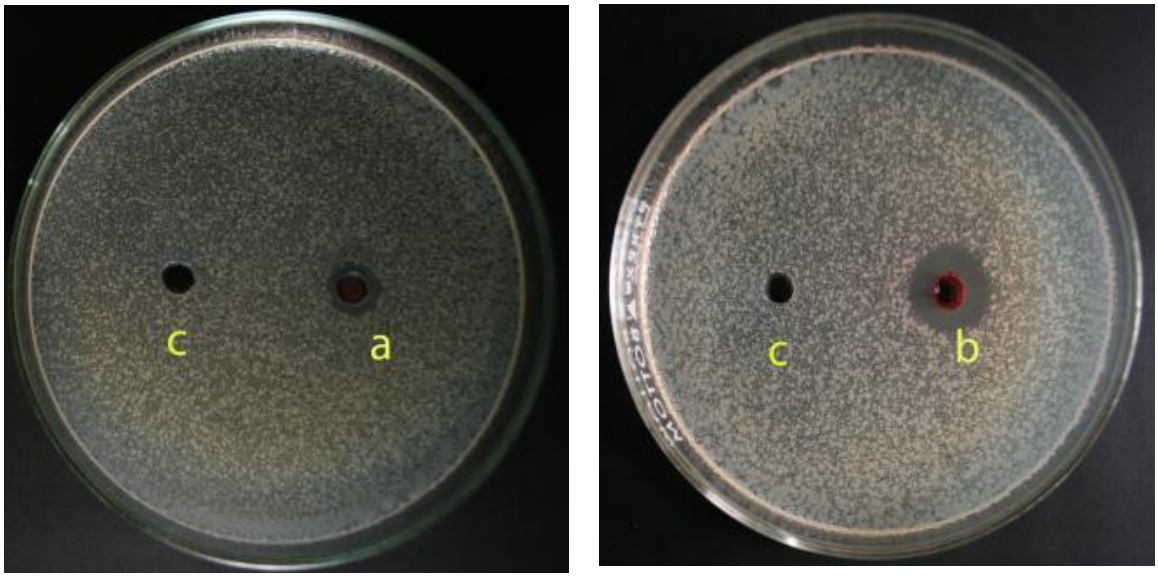

Figure 22 The inhibition zone of *Bacillus subtilis* (cont.) from **a.** Embelin, *Rhinacanthus nasutus* (L.) Kurz (root): **b.** petroleum ether extract; **c.** DMSO

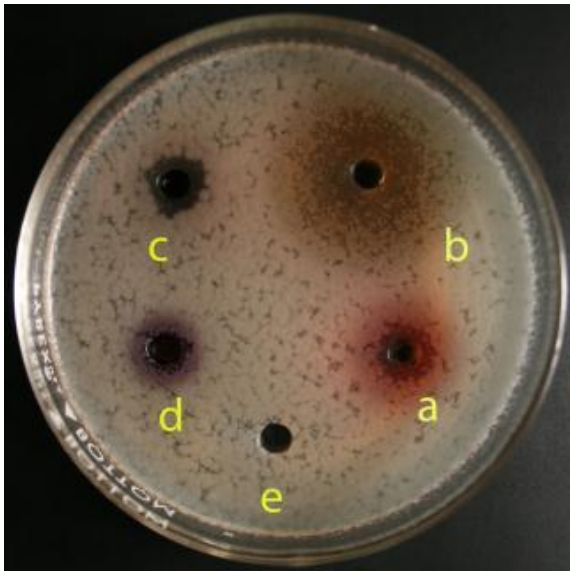

Figure 22 The inhibition zone of *Bacillus subtilis* (cont.) from **a.** Lapachol; **b.** Lawsone; **c.** Juglone; **d.** Alizarin; **e**. DMSO

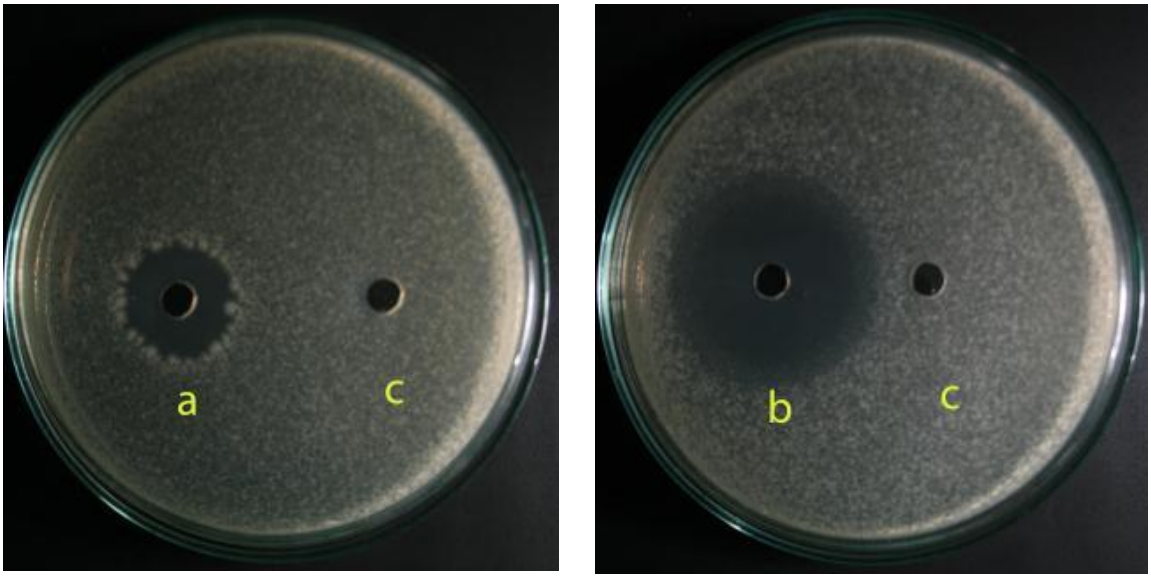

Figure 22 The inhibition zone of *Bacillus subtilis* (cont.) from **a.** Ampicllin sodium; **b.** Amikacin sulfate; **c.** DMSO

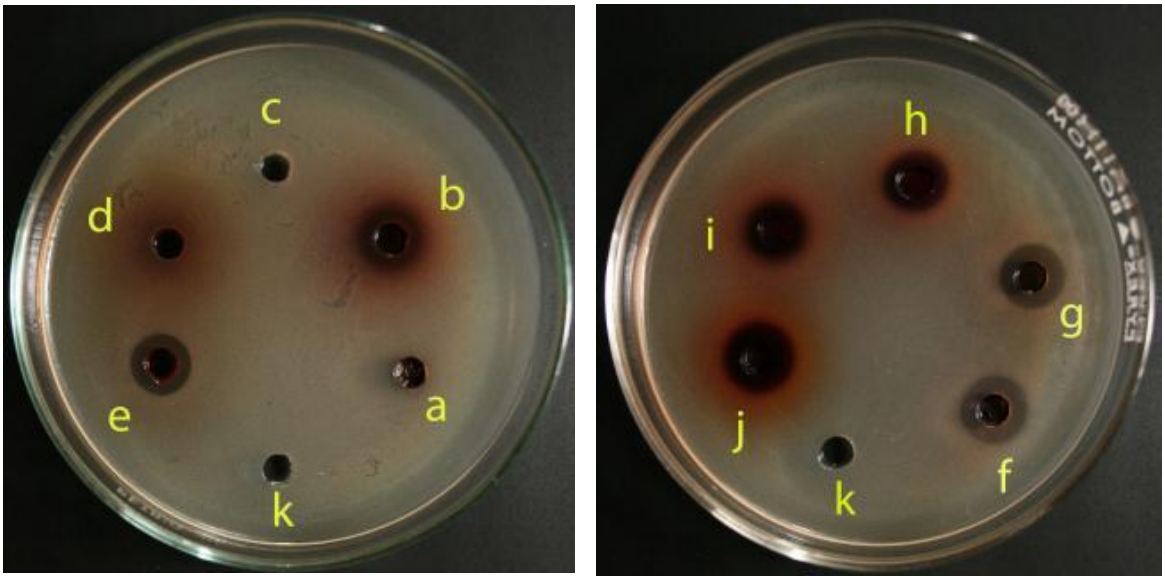

Figure 23 The inhibition zone of *Staphylococcus aureus* from *Xyris indica* L. (flower): **a.** petroleum ether extract, **b.** ethanol extract; *Cassia tora* Linn. (seed): **c.** petroleum ether extract, **d.** ethanol extract; *Rhinacanthus nasutus* (L.) Kurz (root): **e.** ethanol extract; *Rhinacanthus nasutus* (L.) Kurz (aerial part): **f.** petroleum ether extract, **g.** ethanol extract *Morinda elliptica* Ridl. (root): **h.** petroleum ether extract, **i.** ethanol extract; *Morinda citrifolia* L. (root): **j.** petroleum ether extract; **k.** DMSO

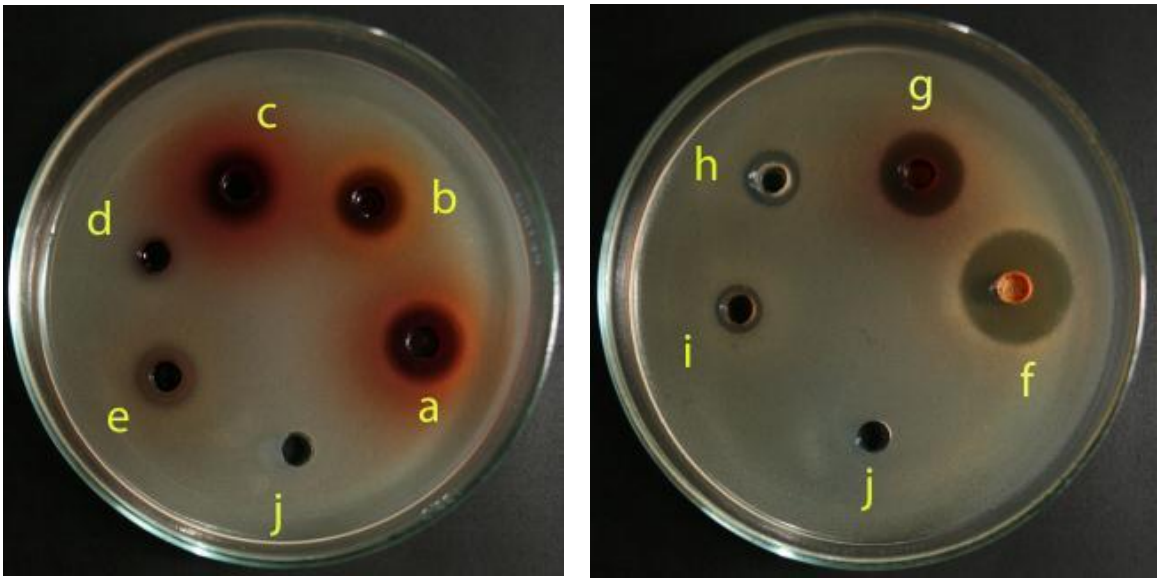

Figure 23 The inhibition zone of *Staphylococcus aureus* (cont.) from *Morinda citrifolia* L. (root): **a.** ethanol extract; *Morinda coreia* Ham. (root): **b.** petroleum ether extract, **c.** ethanol extract; *Ardisia elliptica* Thunb. (fruit): **d.** petroleum ether extract, **e.** ethanol extract; *Eleutherine Americana* (Aubl.) (bulb): **f.** petroleum ether extract, **g.** ethanol extract; *Nigella sativa* Linn. (seed): **h.** petroleum ether extract, **i.** ethanol extract; **j.** DMSO

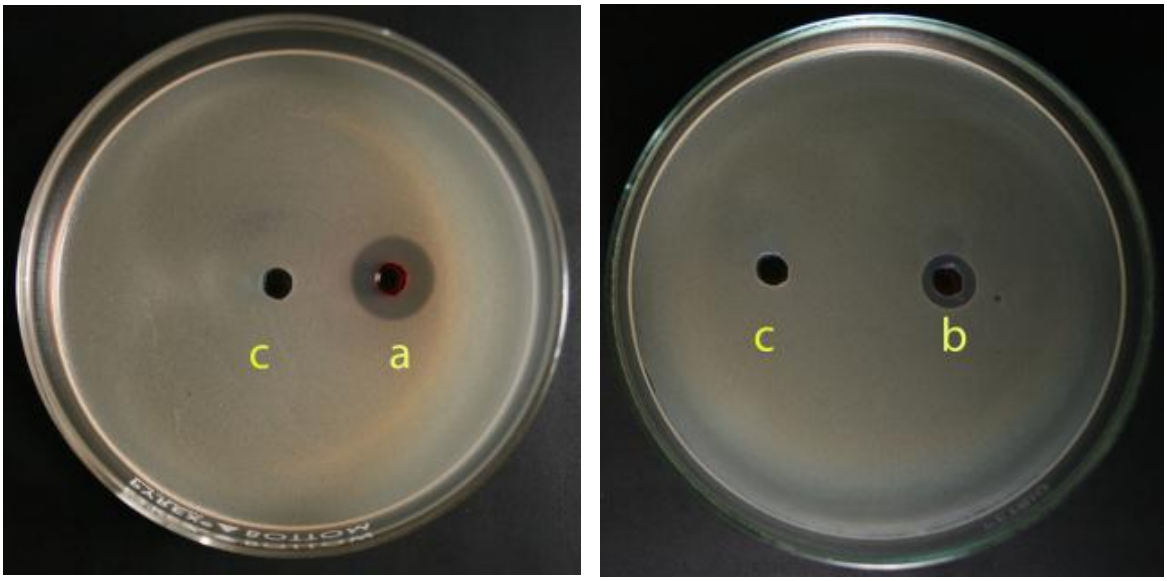

Figure 23 The inhibition zone of *Staphylococcus aureus* (cont.) from *Rhinacanthus nasutus* (L.) Kurz (root): **a.** petroleum ether extract, **b.** Embelin; **c.** DMSO

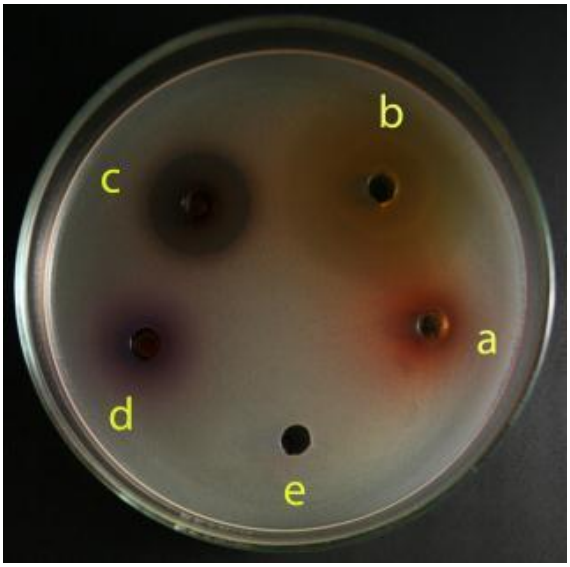

Figure 23 The inhibition zone of *Staphylococcus aureus* (cont.) from **a.** Lapachol; **b.** Lawsone; **c.** Juglone; **d.** Alizarin; **e**. DMSO

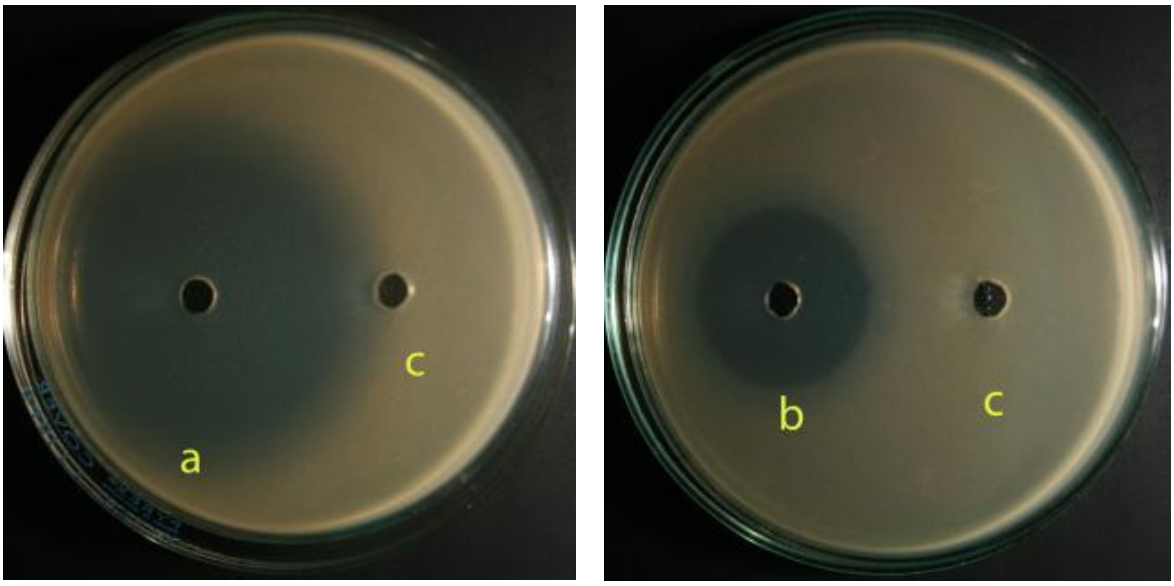

Figure 23 The inhibition zone of *Staphylococcus aureus* (cont.) from **a.** Ampicllin sodium; **b.** Amikacin sulfate; **c.** DMSO

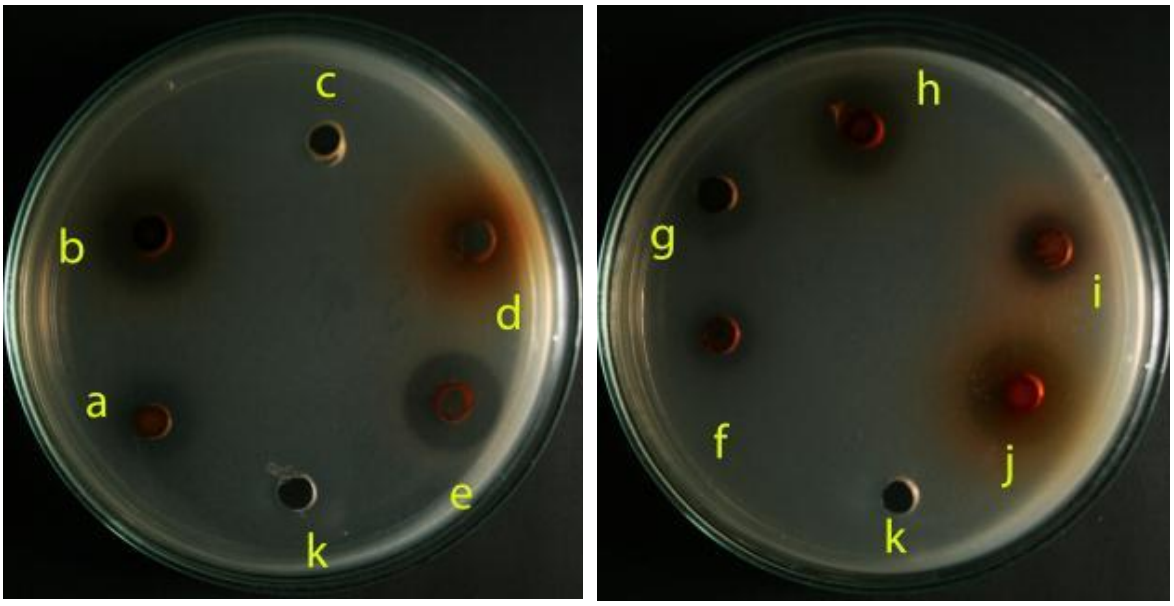

Figure 24 The inhibition zone of *Staphylococcus epidermidis* from *Xyris indica* L. (flower): **a.** petroleum ether extract, **b.** ethanol extract; *Cassia tora* Linn. (seed): **c.** petroleum ether extract, **d.** ethanol extract; *Rhinacanthus nasutus* (L.) Kurz (root): **e.** ethanol extract; *Rhinacanthus nasutus* (L.) Kurz (aerial part): **f.** petroleum ether extract, **g.** ethanol extract; *Morinda elliptica* Ridl. (root): **h.** petroleum ether extract, **i.** ethanol extract; *Morinda citrifolia* L. (root): **j.** petroleum ether extract; **k.** DMSO

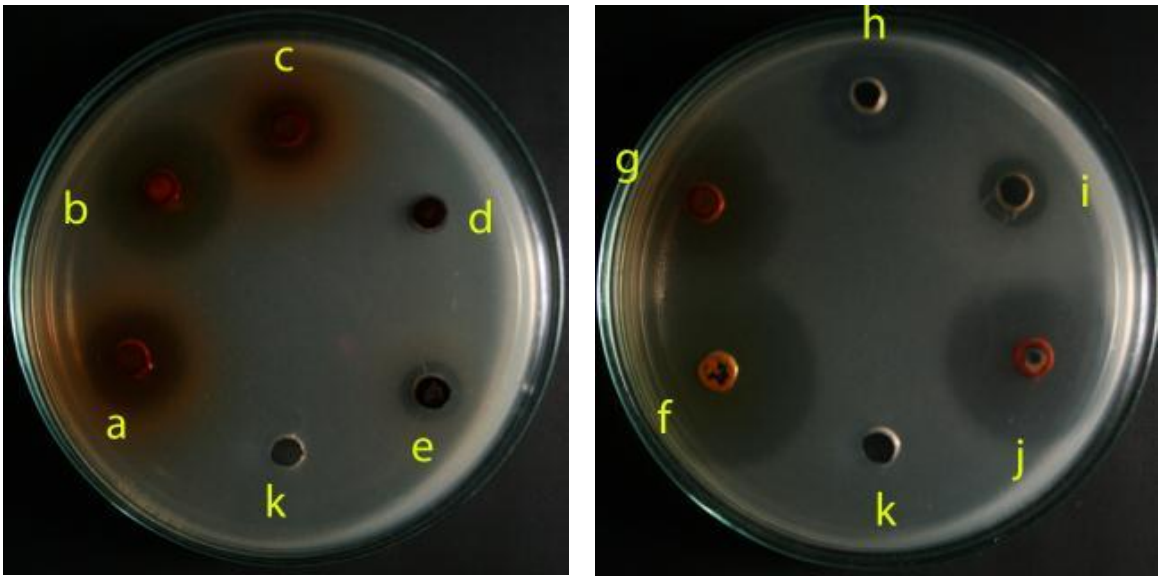

Figure 24 The inhibition zone of *Staphylococcus epidermidis* (cont.) from *Morinda citrifolia* L. (root): **a.** ethanol extract; *Morinda coreia* Ham. (root): **b.** petroleum ether extract, **c.** ethanol extract; *Ardisia elliptica* Thunb. (fruit): **d.** petroleum ether extract, **e.** ethanol extract; *Eleutherine Americana* (Aubl.) (bulb): **f.** petroleum ether extract, **g.** ethanol extract; *Nigella sativa* Linn. (seed): **h.** petroleum ether extract, **i.** ethanol extract; *Rhinacanthus nasutus* (L.) Kurz (root): **j.** petroleum ether extract; **k.** DMSO

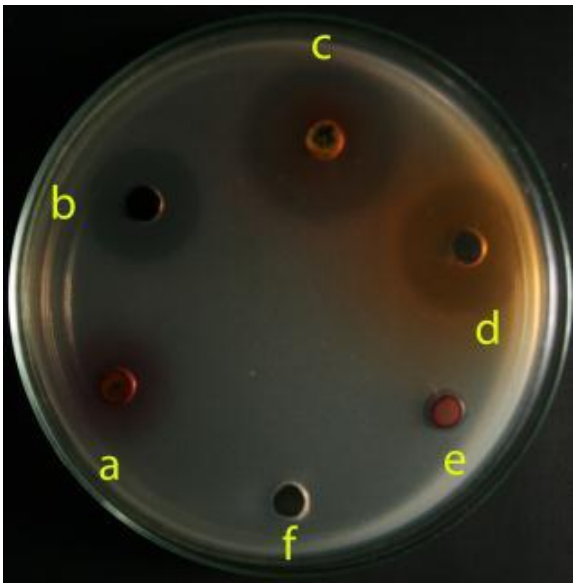

Figure 24 The inhibition zone of *Staphylococcus epidermidis* (cont.) from **a.** Alizarin; **b.** Juglone; **c.** Lawsone; **d.** Lapachol; **e.** Embelin; **f.** DMSO

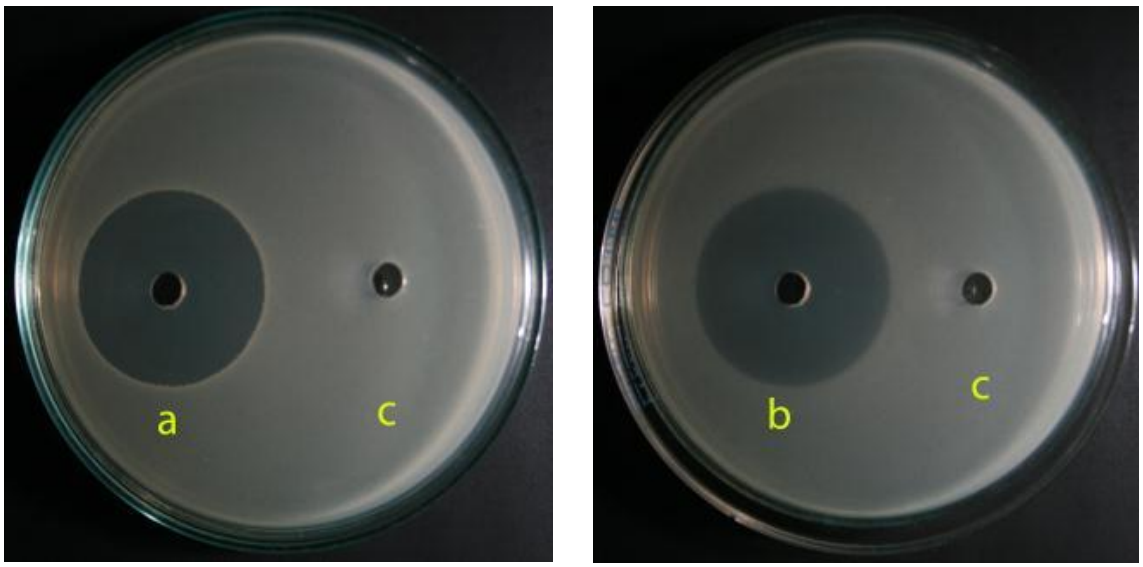

Figure 21 The inhibition zone of *Staphylococcus epidermidis* (cont.) from **a.** Ampicllin sodium; **b.** Amikacin sulfate; **c.** DMSO

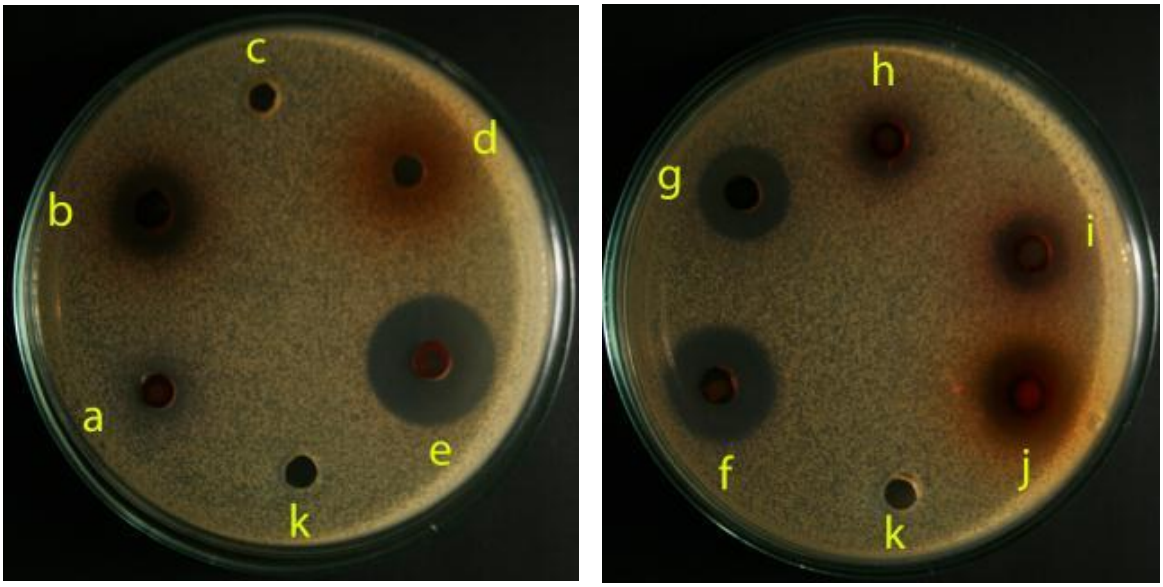

Figure 25 The inhibition zone of *Micrococcus luteus* from *Xyris indica* L. (flower): **a.** petroleum ether extract, **b.** ethanol extract; *Cassia tora* Linn. (seed): **c.** petroleum ether extract, **d.** ethanol extract; *Rhinacanthus nasutus* (L.) Kurz (root): **e.** ethanol extract; *Rhinacanthus nasutus* (L.) Kurz (aerial part): **f.** petroleum ether extract, **g.** ethanol extract; *Morinda elliptica* Ridl. (root): **h.** petroleum ether extract, **i.** ethanol extract; *Morinda citrifolia* L. (root): **j.** petroleum ether extract; **k.** DMSO

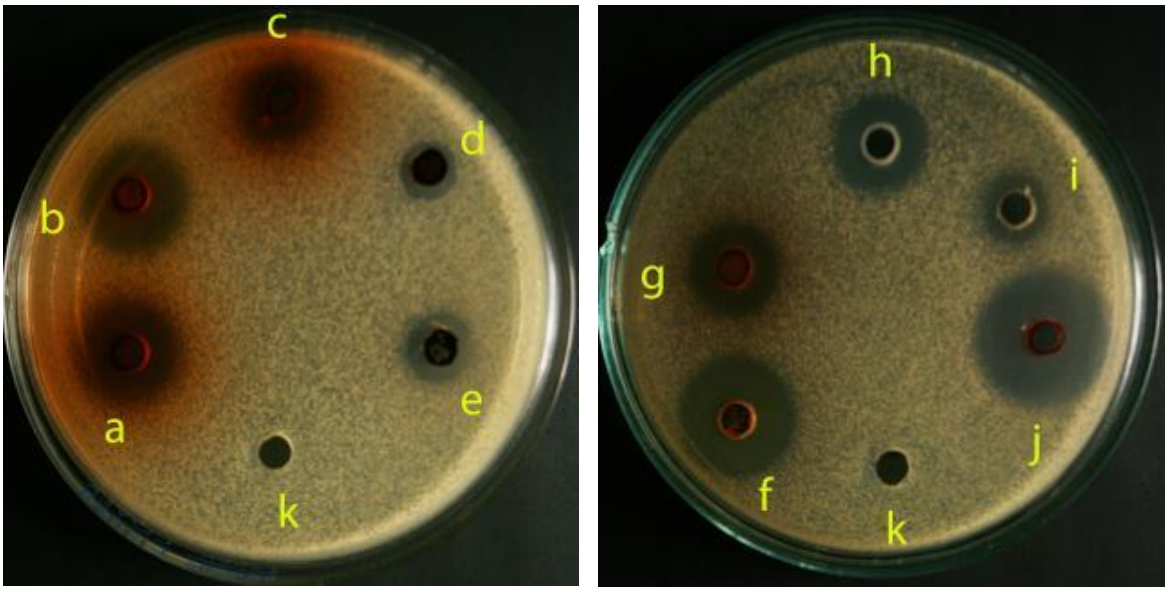

Figure 25 The inhibition zone of *Micrococcus luteus* (cont.) from *Morinda citrifolia* L. (root): **a.** ethanol extract; *Morinda coreia* Ham. (root): **b.** petroleum ether extract, **c.** ethanol extract; *Ardisia elliptica* Thunb. (fruit): **d.** petroleum ether extract, **e.** ethanol extract; *Eleutherine Americana* (Aubl.) (bulb): **f.** petroleum ether extract, **g.** ethanol extract; *Nigella sativa* Linn. (seed): **h.** petroleum ether extract, **i.** ethanol extract; *Rhinacanthus nasutus* (L.) Kurz (root): **j.** petroleum ether extract; **k.** DMSO

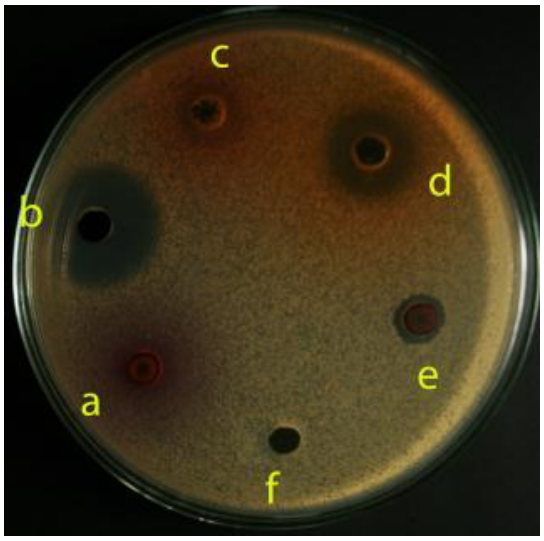

Figure 25 The inhibition zone of *Micrococcus luteus* (cont.) from **a.** Alizarin; **b.** Juglone; **c.** Lawsone; **d.** Lapachol; **e.** Embelin; **f.** DMSO

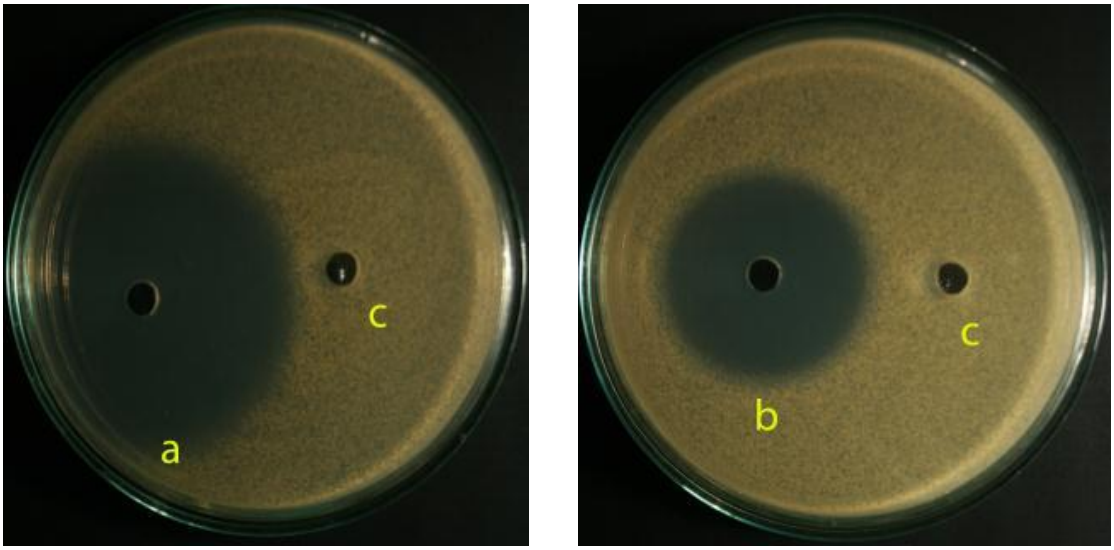

Figure 25 The inhibition zone of *Micrococcus luteus* (cont.) from **a.** Ampicllin sodium; **b.** Amikacin sulfate; **c.** DMSO

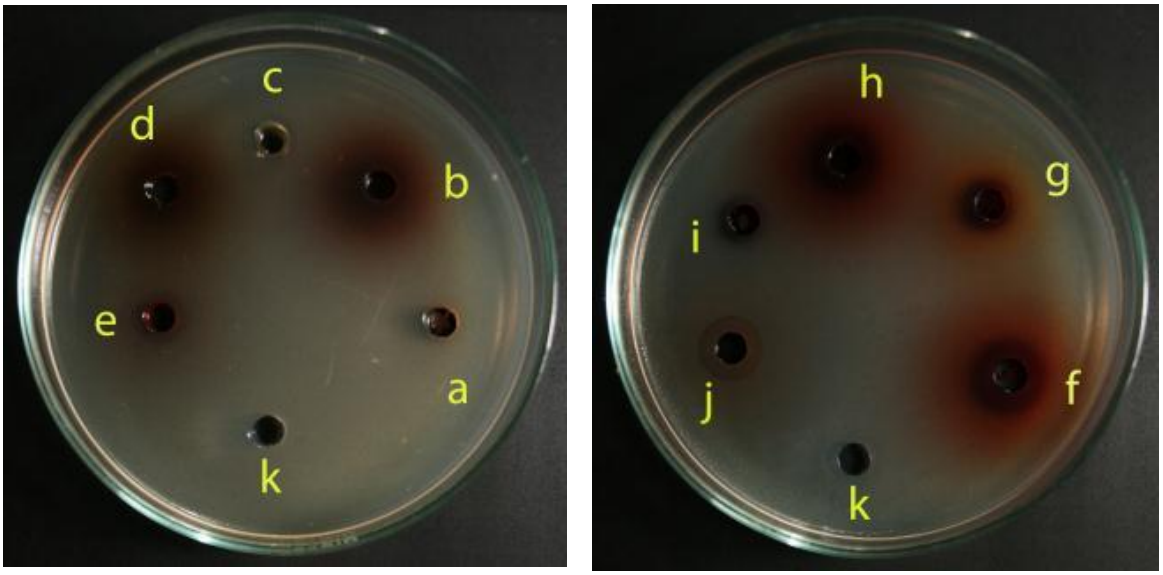

Figure 26 The inhibition zone of *Escherichia coli* from *Xyris indica* L. (flower) from **a.** petroleum ether extract, **b.** ethanol extract; *Cassia tora* Linn. (seed): **c.** petroleum ether extract, **d.** ethanol extract; *Rhinacanthus nasutus* (L.) Kurz (root): **e.** ethanol extract; *Morinda citrifolia* L. (root): **f.** ethanol extract; *Morinda coreia* Ham. (root): **g.** petroleum ether extract, **h.** ethanol extract; *Ardisia elliptica* Thunb. (fruit): **i.** petroleum ether extract, **j.** ethanol extract; **k.** DMSO

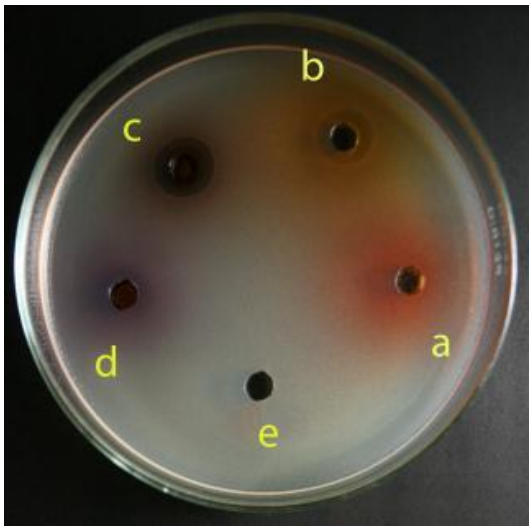

Figure 26 The inhibition zone of *Escherichia coli* (cont.) from **a.** Lapachol; **b.** Lawsone; **c.** Juglone; **d.** Alizarin; **e**. DMSO

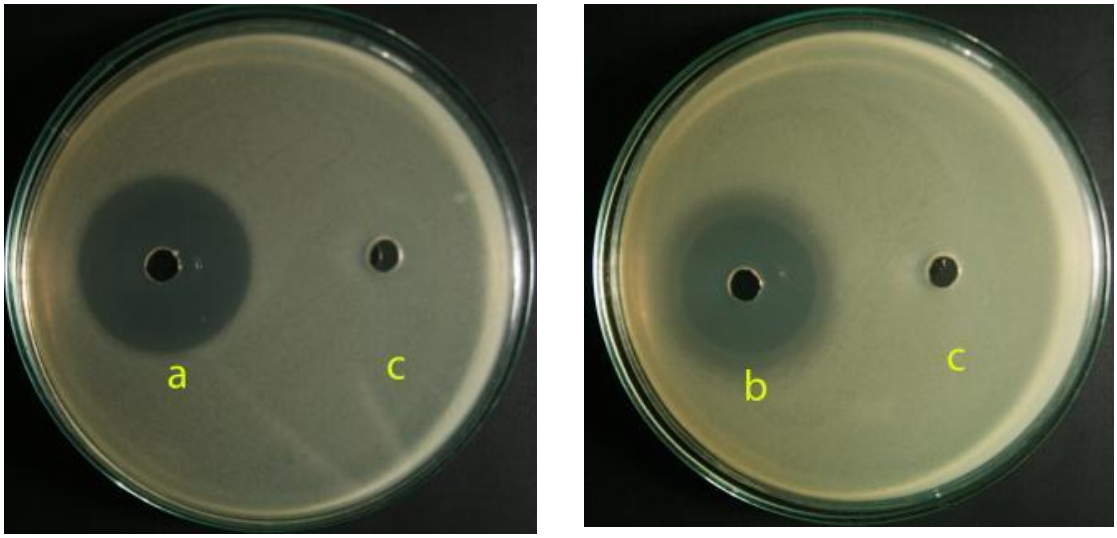

Figure 26 The inhibition zone of *Escherichia coli* (cont.) from **a.** Ampicllin sodium; **b.** Amikacin sulfate; **c.** DMSO

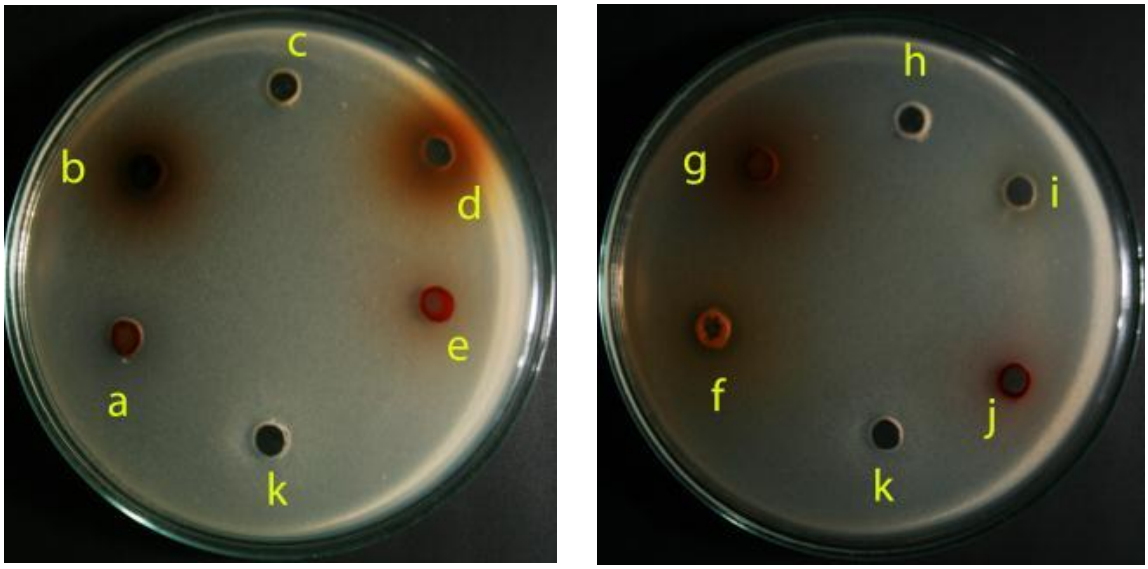

Figure 27 The inhibition zone of *Enterobacter aerogenes* from *Xyris indica* L. (flower): **a.** petroleum ether extract, **b.** ethanol extract; *Cassia tora* Linn. (seed): **c.** petroleum ether extract, **d.** ethanol extract; *Rhinacanthus nasutus* (L.) Kurz (root): **e.** ethanol extract; *Eleutherine Americana* (Aubl.) (bulb): **f.** petroleum ether extract, **g.** ethanol extract; *Nigella sativa* Linn. (seed): **h.** petroleum ether extract, **i.** ethanol extract; *Rhinacanthus nasutus* (L.) Kurz (root): **j.** petroleum ether extract; **k.** DMSO

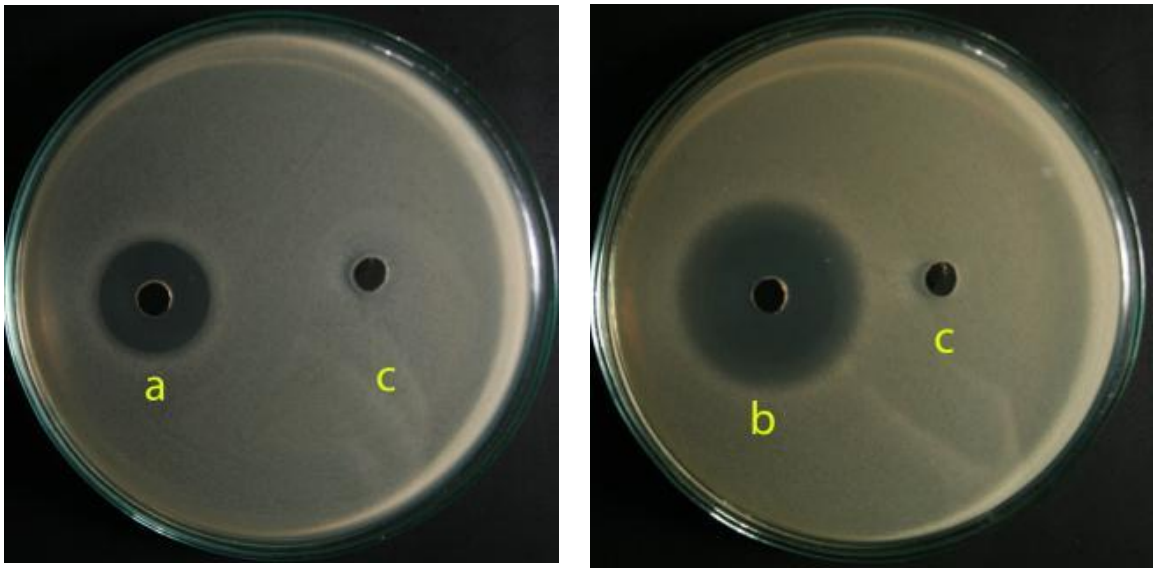

Figure 27 The inhibition zone of *Enterobacter aerogenes* (cont.) from **a.** Ampicllin sodium; **b.** Amikacin sulfate; **c.** DMSO

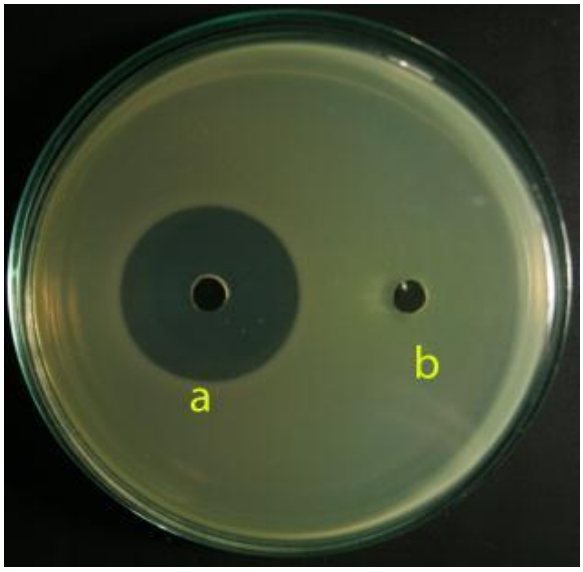

Figure 28 The inhibition zone of *Pseudomonas aeruginosa* from **a.** Amikacin sulfate; **b.** DMSO

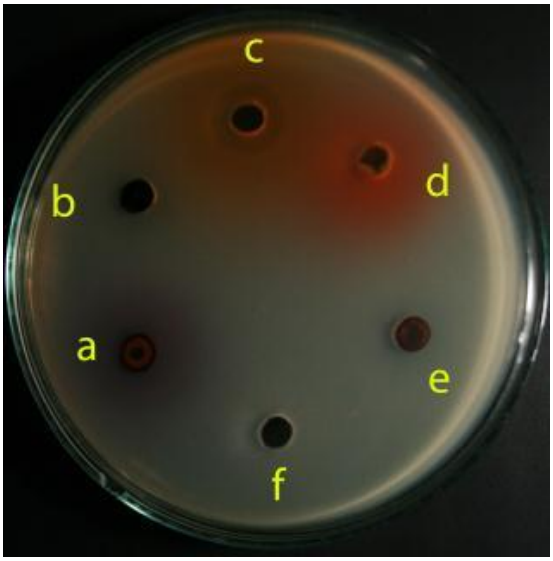

Figure 29 The inhibition zone of *Salmonella typhi* from **a.** Alizarin; **b.** Juglone; **c.** Lawsone; **d.** Lapachol; **e.** Embelin; **f.** DMSO

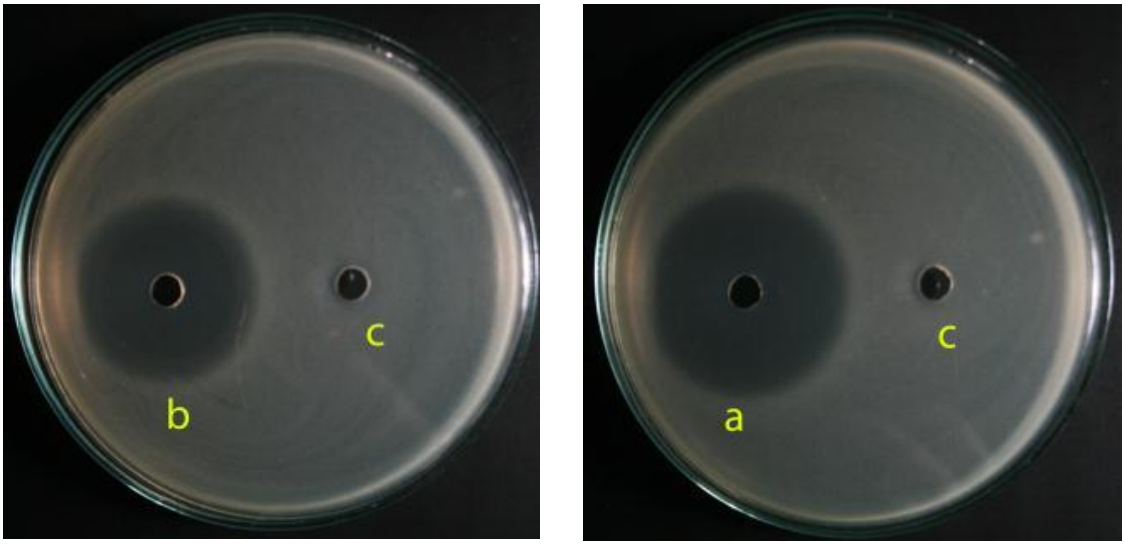

Figure 29 The inhibition zone of *Salmonella typhi* (cont.) from **a.** Ampicllin sodium; **b.** Amikacin sulfate; **c.** DMSO

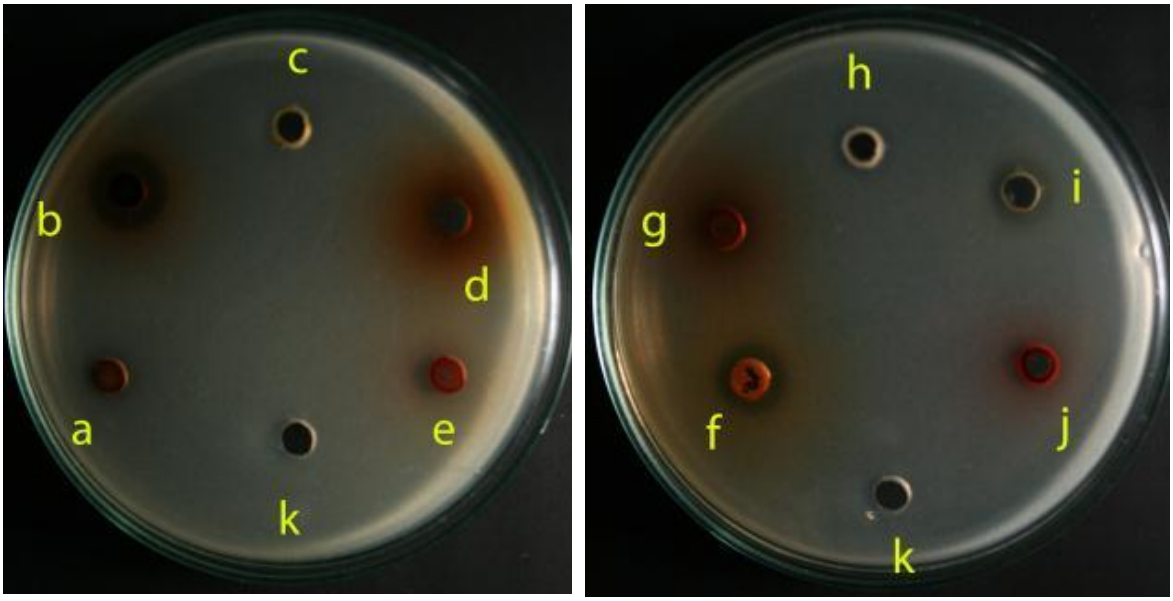

Figure 30 The inhibition zone of *Salmonella typhimurium* from *Xyris indica* L. (flower): **a.** petroleum ether extract, **b.** ethanol extract; *Cassia tora* Linn. (seed): **c.** petroleum ether extract, **d.** ethanol extract; *Rhinacanthus nasutus* (L.) Kurz (root): **e.** ethanol extract;*Eleutherine Americana* (Aubl.) (bulb): **f.** petroleum ether extract, **g.** ethanol extract; *Nigella sativa* Linn. (seed): **h.** petroleum ether extract, **i.** ethanol extract; *Rhinacanthus nasutus* (L.) Kurz (root): **j.** petroleum ether extract; **k.** DMSO

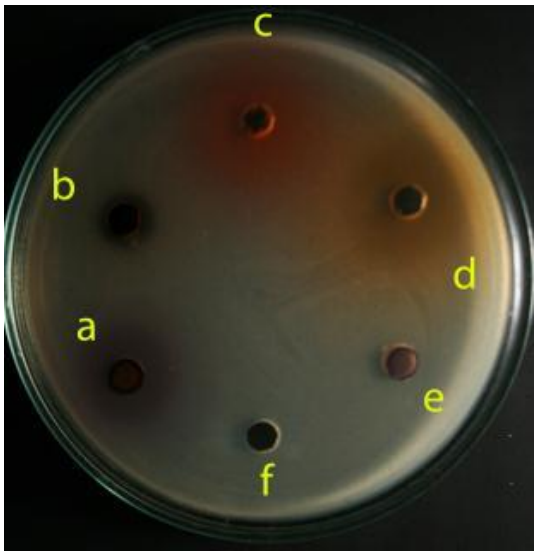

Figure 30 The inhibition zone of *Salmonella typhimurium* (cont.) from **a.** Alizarin; **b.** Juglone; **c.** Lapachol; **d.** Lawsone; **e.** Embelin; **f.** DMSO

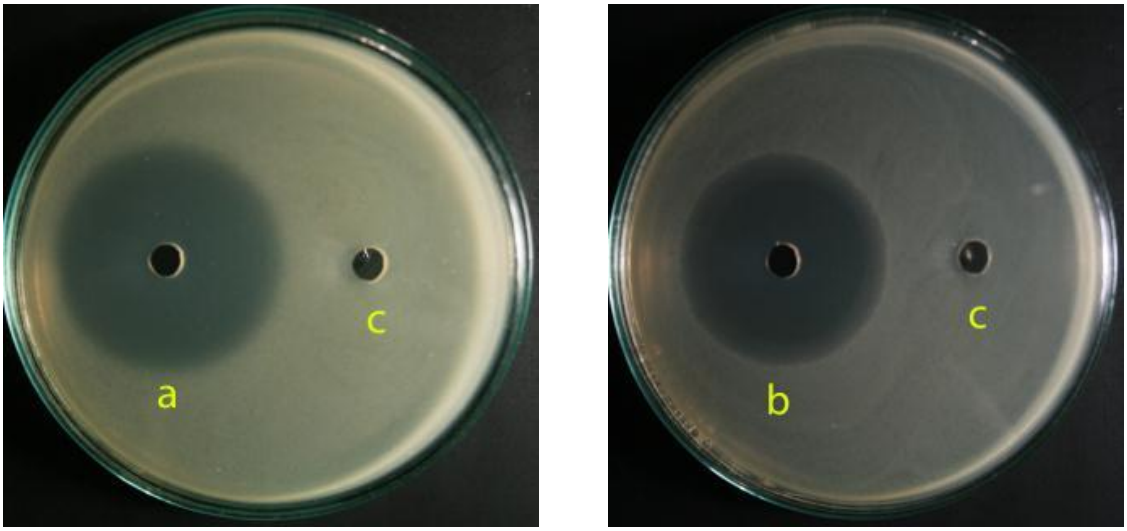

Figure 30 The inhibition zone of *Salmonella typhimurium* (cont.) from **a.** Ampicllin sodium; **b.** Amikacin sulfate; **c.** DMSO

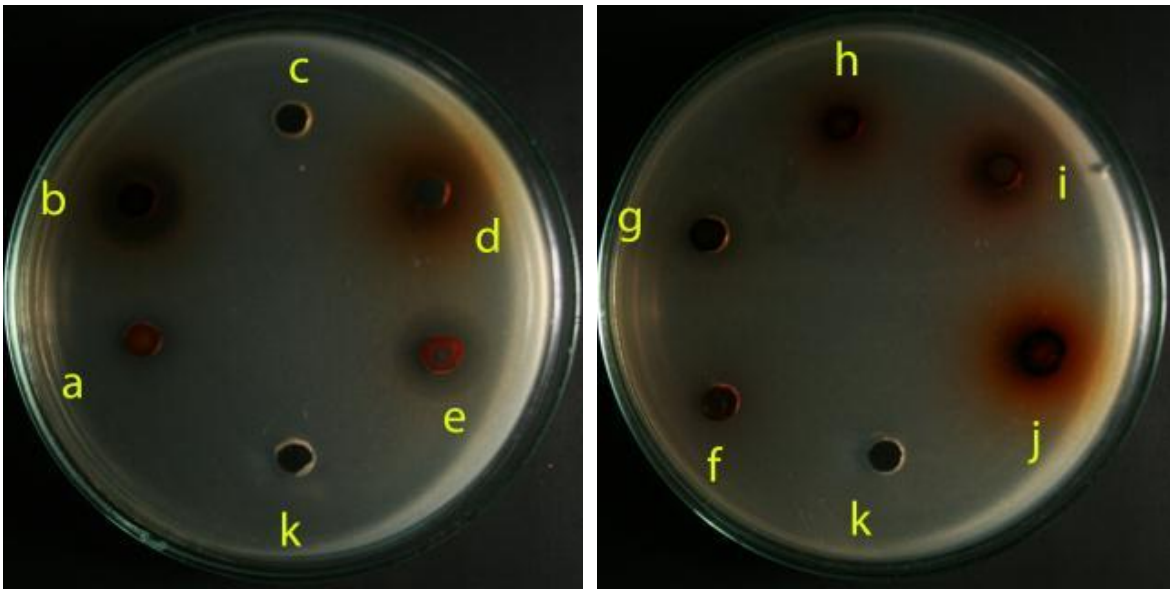

Figure 31 The inhibition zone of *Shigella sp*. from *Xyris indica* L. (flower) from **a.** petroleum ether extract, **b.** ethanol extract; *Cassia tora* Linn. (seed): **c.** petroleum ether extract, **d.** ethanol extract; *Rhinacanthus nasutus* (L.) Kurz (root): **e.** ethanol extract; *Rhinacanthus nasutus* (L.) Kurz (aerial part): **f.** petroleum ether extract, **g.** ethanol extract; *Morinda elliptica* Ridl. (root): **h.** petroleum ether extract, **i.** ethanol extract; *Morinda citrifolia* L. (root): **j.** petroleum ether extract; **k.** DMSO

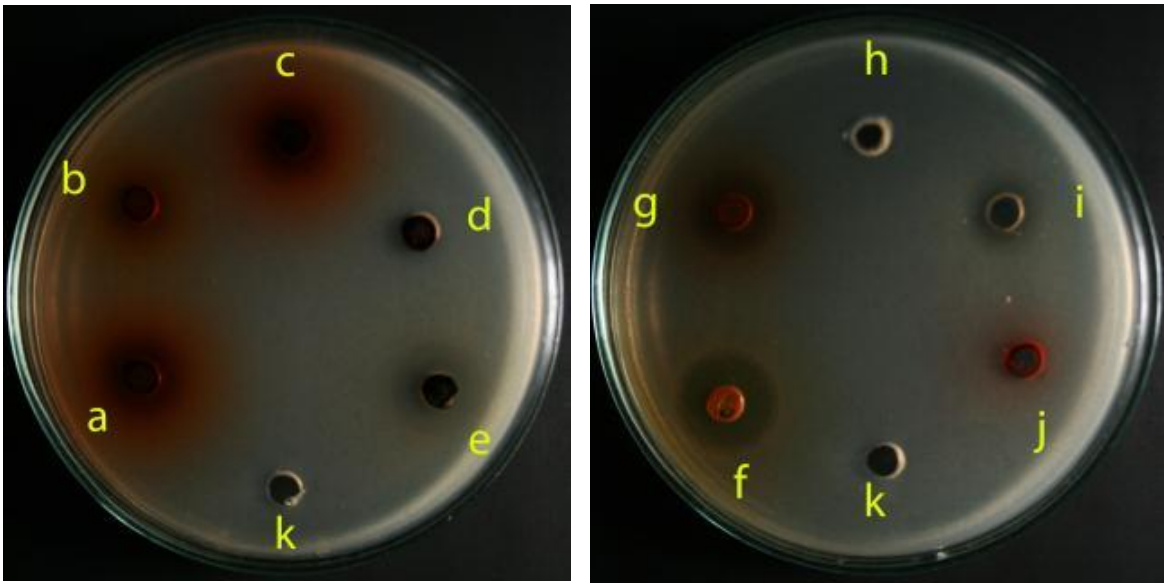

Figure 31 The inhibition zone of *Shigella sp.* (cont.) from *Morinda citrifolia* L. (root): **a.** ethanol extract; *Morinda coreia* Ham. (root): **b.** petroleum ether extract, **c.** ethanol extract; *Ardisia elliptica* Thunb. (fruit): **d.** petroleum ether extract, **e.** ethanol extract; *Eleutherine Americana* (Aubl.) (bulb): **f.** petroleum ether extract, **g.** ethanol extract; *Nigella sativa* Linn. (seed): **h.** petroleum ether extract, **i.** ethanol extract; *Rhinacanthus nasutus* (L.) Kurz (root): **j.** petroleum ether extract; **k.** DMSO

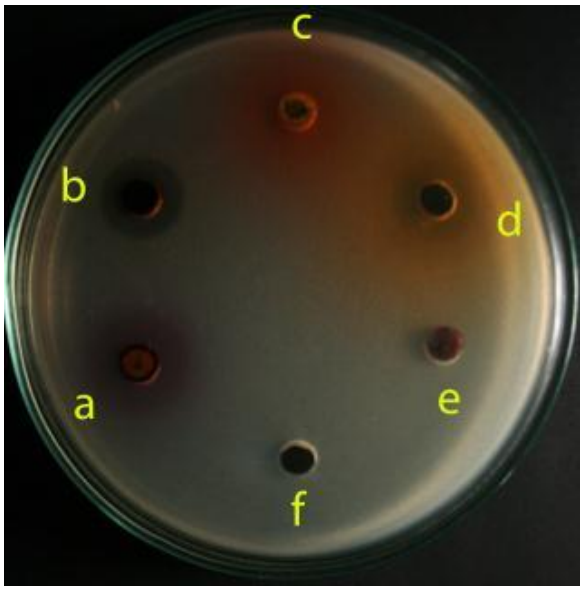

Figure 31 The inhibition zone of *Shigella sp.* (cont.) from **a.** Alizarin; **b.** Juglone; **c.** Lapachol; **d.** Lawsone; **e.** Embelin; **f.** DMSO

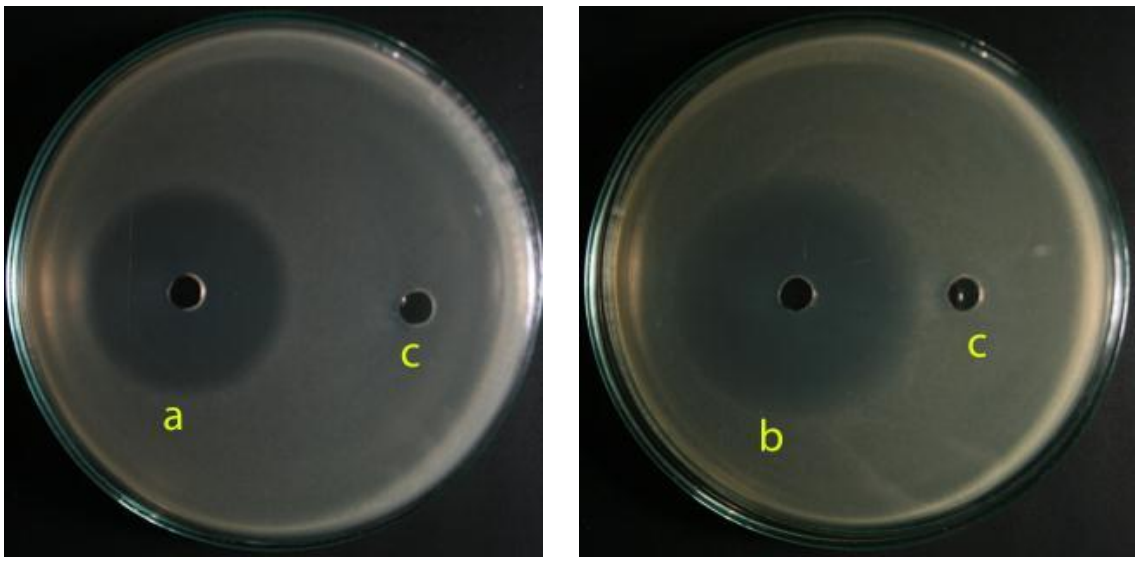

Figure 31 The inhibition zone of *Shigella sp.* (cont.) from **a.** Ampicllin sodium; **b.** Amikacin sulfate; **c.** DMSO

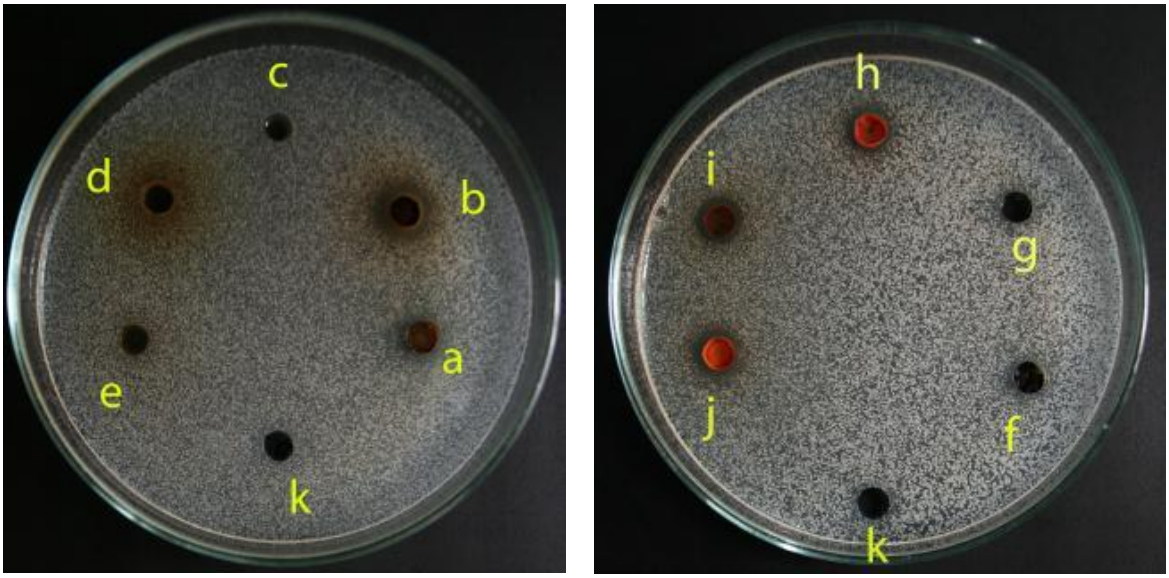

Figure 32 The inhibition zone of *Candida albicans* from *Xyris indica* L. (flower): **a.** petroleum ether extract, **b.** ethanol extract; *Cassia tora* Linn. (seed): **c.** petroleum ether extract, **d.** ethanol extract; *Rhinacanthus nasutus* (L.) Kurz (root): **e.** ethanol extract; *Rhinacanthus nasutus* (L.) Kurz (aerial part): **f.** petroleum ether extract, **g.** ethanol extract; *Morinda elliptica* Ridl. (root): **h.** petroleum ether extract, **i.** ethanol extract; *Morinda citrifolia* L. (root): **j.** petroleum ether extract; **k.** DMSO

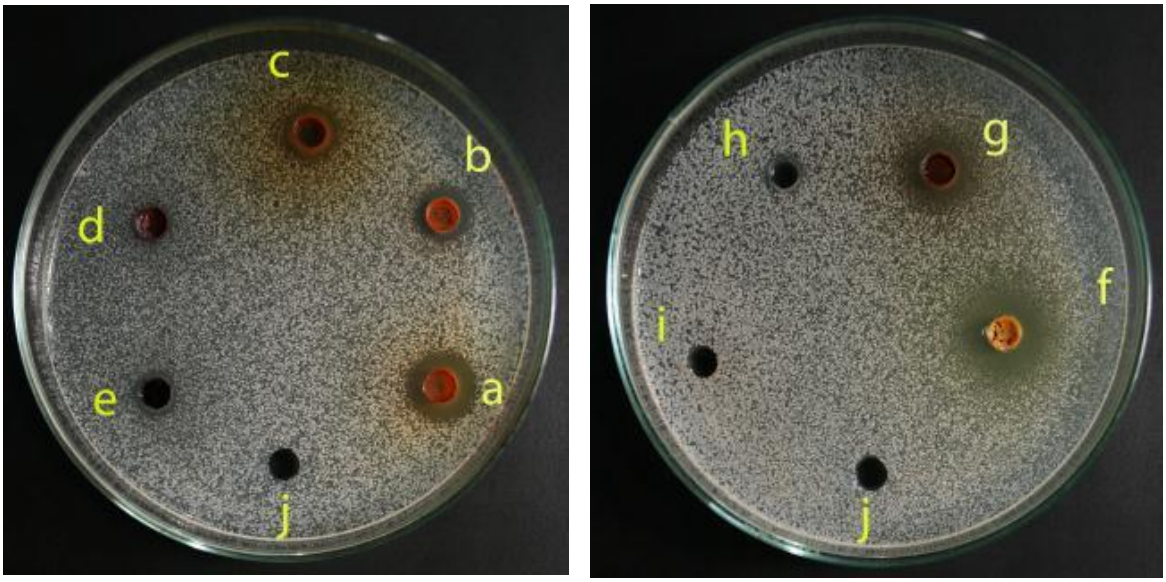

Figure 32 The inhibition zone of *Candida albicans* (cont.) from *Morinda citrifolia* L. (root): **a.** ethanol extract; *Morinda coreia* Ham. (root): **b.** petroleum ether extract, **c.** ethanol extract; *Ardisia elliptica* Thunb. (fruit): **d.** petroleum ether extract, **e.** ethanol extract; *Eleutherine Americana* (Aubl.) (bulb): **f.** petroleum ether extract, **g.** ethanol extract; *Nigella sativa* Linn. (seed): **h.** petroleum ether extract, **i.** ethanol extract; **j.** DMSO

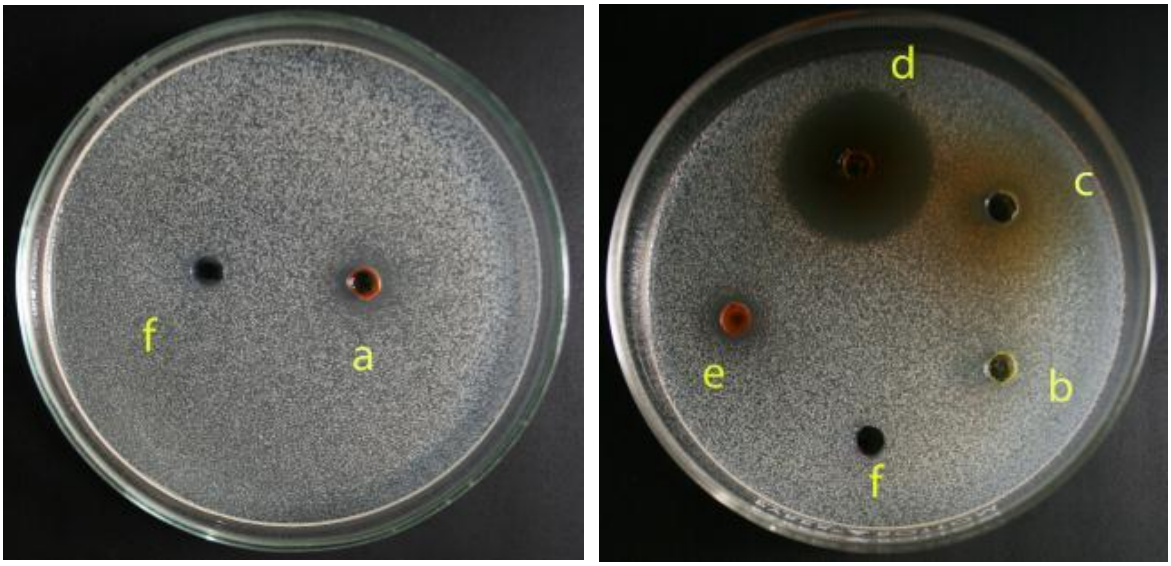

Figure 32 The inhibition zone of *Candida albicans* (cont.) from *Rhinacanthus nasutus* (L.) Kurz (root): **a.** petroleum ether extract; **b.** Lapachol; **c.** Lawsone; **d.** Juglone; **e.** Alizarin; **f.** DMSO

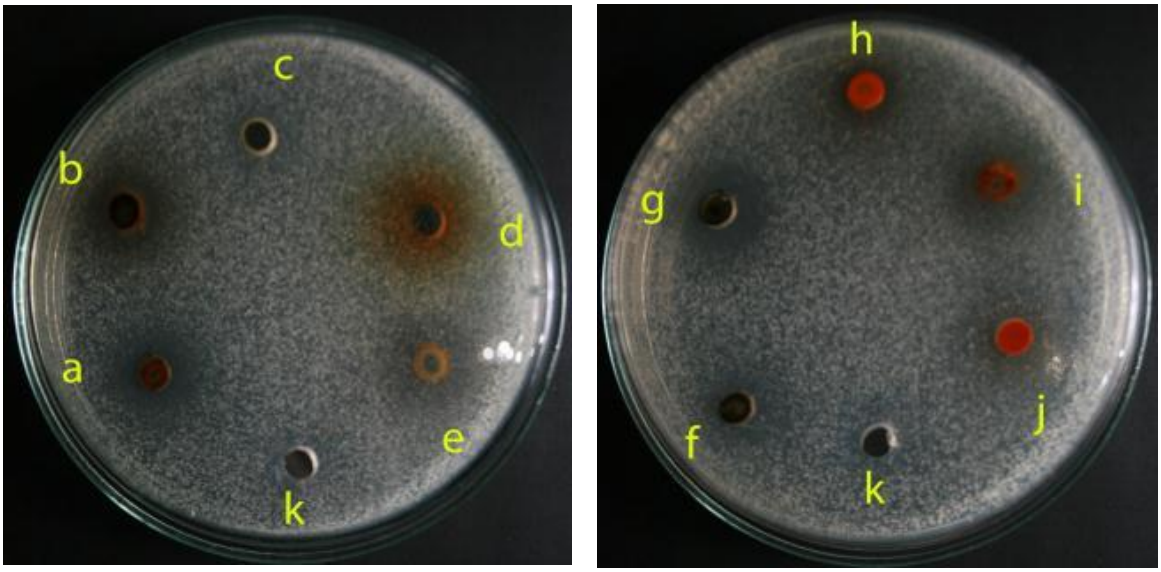

Figure 33 The inhibition zone of *Saccharomyces cerevisiae* from *Xyris indica* L. (flower): **a.** petroleum ether extract, **b.** ethanol extract; *Cassia tora* Linn. (seed): **c.** petroleum ether extract, **d.** ethanol extract; *Rhinacanthus nasutus* (L.) Kurz (root): **e.** ethanol extract; *Rhinacanthus nasutus* (L.) Kurz (aerial part): **f.** petroleum ether extract, **g.** ethanol extract; *Morinda elliptica* Ridl. (root): **h.** petroleum ether extract, **i.** ethanol extract; *Morinda citrifolia* L. (root): **j.** petroleum ether extract; **k.** DMSO

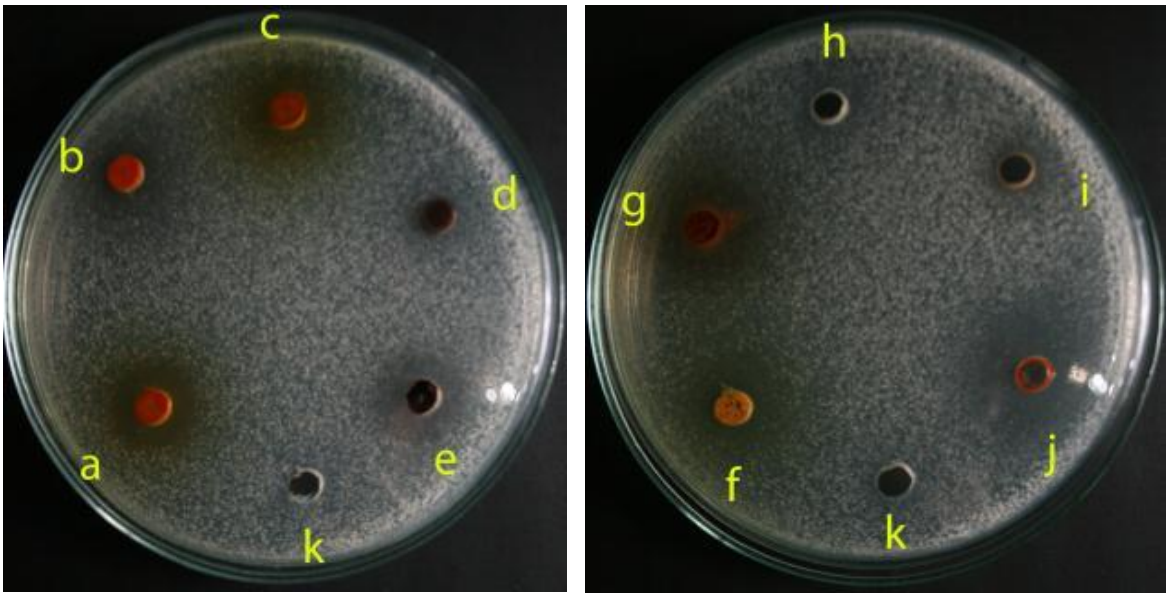

Figure 33 The inhibition zone of *Saccharomyces cerevisiae* (cont.) from *Morinda citrifolia* L. (root): **a.** ethanol extract; *Morinda coreia* Ham. (root): **b.** petroleum ether extract, **c.** ethanol extract; *Ardisia elliptica* Thunb. (fruit): **d.** petroleum ether extract, **e.** ethanol extract; *Eleutherine Americana* (Aubl.) (bulb): **f.** petroleum ether extract, **g.** ethanol extract; *Nigella sativa* Linn. (seed): **h.** petroleum ether extract, **i.** ethanol extract; *Rhinacanthus nasutus* (L.) Kurz (root): **j.** petroleum ether extract; **k.** DMSO

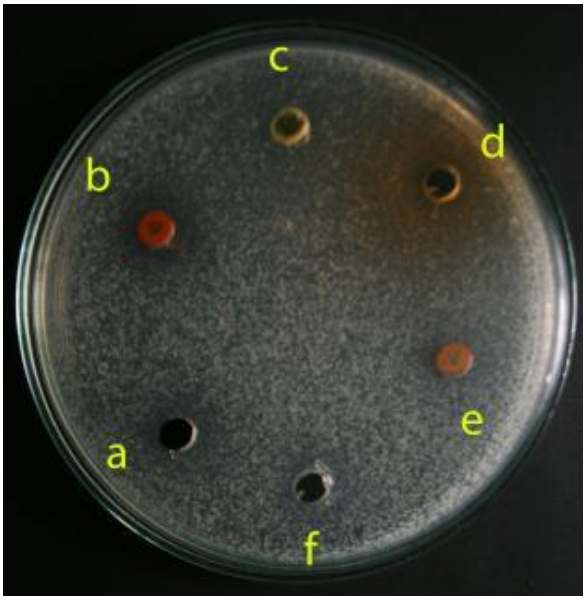

Figure 33 The inhibition zone of *Saccharomyces cerevisiae* (cont.) from **a.** Juglone; **b.** Alizarin; **c.** Lapachol; **d.** Lawsone; **e.** Embelin; **f.** DMSO

VITA

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Proceeding:

Chansukh K., Palanuvej C. and Ruangrungsi N. Antibacreial activities of selected Thai medicinal plants bearing quinonids. The 37th Congress on Science and Technology of Thailand (STT 37): Creative Sciences for Creating the Future. The Science Society of Thailand under the Patronage of His Majesty the King and Faculty of Science, Mahidol University, Thailand, October 11-12. 2011.