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Mangrove *Avicennia marina* of Yanbu, Saudi Arabia: GC-MS Constituents and Mosquito Repellent Activities

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ABSTRACT

A phytochemical analysis of *Avicennia marina* has been investigated by GC-MS. *Avicennia marina* leaves, seeds, flowers, stems indicate the presence of, steroids, tannins, glycosides, carbohydrates saponnins, sterols, terpenoids and phenols. The *Avicennia marina* revealed the presence of active medicinal constituents by GC-MS. This study also helped to identify the formula and structure of biomolecular therapy which can be used as drugs. *Avicennia marina* was tested for their mosquitocidal activities. The toxicities were examined against the lab. Strain of 3rd instar larvae of *Culex pipiens*. The results showed that extracts of seeds and leaves of *Avicennia marina* were more efficient than other parts against third instar larvae of *Culex pipiens* mosquito. In view of these results, the purified active compounds from the most effective samples found in our studies could be effective in killing mosquito larvae or repelling adult female mosquitoes in an economical and safe manner.

INTRODUCTION

The Mangroves are trees and shrubs which raise within saline seaside in the tropics and subtropics region **(Saenger, 2002)**. **Kathiresan and Bingham (2001)** recognised 65 mangrove species in 22 genera and 16 families. Current reports demonstrate that the plant extract includes a tremendous hepatoprotective effect **(Miles *et al.*, 1999)**. Currently, it was highly suggested that mangroves are highly recommended as a precious resource for chemical constituents with prospective therapeutic and agricultural values **(Miles *et al.*, 1999).** Studies currently revealed the finding of various narrative compounds with potential therapeutic importance for the improvement of novel chemotherapy from mangrove plants.

Avicennia marina (Forssk.) Vierh. (Avicenniaceae) has got much more interest in figuring out its essential chemical components. A napthoforan compound with phytoalexin activity has been isolated **(Sutton *et al.*, 1985 and Miles *et al.*, 1999).** Fatty acids, sterols and hydrocarbons had been studied in consideration to their chemotaxonomic implication in some mangroves including *A. marina* **(Hogg and Gillan 1984).** The presence or absence of an iridoid glucoside 2′-cinnamoyl mussaenosidic acid from *A. marina* extracts is used in sub specific chemotaxonomy **(Bousquet-Mélou and Fauvel 1998).** It is a traditional medicinal plant used mainly against ulcer, skin disease, snake-bites and asthma, paralysis and rheumatism. The usage of the Indian mangrove as a folk medication for tumours and boils. It was found that the resinous substance that extracted from the Indian mangrove bark are successfully utilised as a contraceptive and can safelybe used for a long time without any considering side effects **(Thirunavukkarasu *et al.*, 2010)**. Over the past few years, synthetic compounds were applied with large amounts to manage insect pests **(Thangam and Kathiresan, 1990).** The chemical pesticides are often non-target specific and may result in environmental deterioration because of their continual nature. Internationally there is a prolonged reputation of plant products being utilised for their insecticidal or repellent properties. Hence natural insecticides were realised to be eco-friendly and are given preference **(Nazar *et al*., 2009).**

Through this perspective, many terrestrial plants have been formerly screened for mosquito larvicidal and/or repellent activities **(Thangam and Kathiresan, 1990, 1994).** The plant extracts have recently gained an importance of insect control, being considered safe to the environment and less hazardous to non-target biota (**Gajendran and Ragupathy, 2003).** Intensive work was conducted on the biological activity of plant extracts as natural sources of insecticides **(Saleh *et al.*, 1983** and **Thangam and kathiresan 1993)**. However, the plant extracts may act as toxicants or repellents **(Su, and Harvart, 1981; Sharma and Dhiman, 1993)** or act as insect growth regulators **(Bowers *et al*., 1972).** Cytotoxicity, anti-candidal activities, anti-bacteriophage as well as antibacterial were previously in vitro assessed in the ethanol and aqueous extracts of the mangrove *Avicennia marina* **(Khafagi *et al.*, 2003).** They found that the aqueous extracts of roots and shoot seedlings exhibited an anti-bacteriophage activity using coliphage against *Escherichia coli* showing antiviral activity. Recent evidence suggests that the aqueous extracts also displayed average cytotoxicity against the larvae of the brine shrimp *Artemia salina*, which demonstrated antimalarial and anti-plasmodial activities **(Khafagi *et al,*. 2003).** The seeds were found to be the most efficient followed by leaves and flowers. Experts have highly suggested that mangroves might be of interest being a precious supply for chemical components with prospective therapeutic and agricultural values **(Miles *et al.*, 1999).** Chemical constituents as well as biological activities and medicinal importance of the mangrove *Avicennia marina* were recently reviewed **(Thatoi *et al.*, 2016**). Analysis has led so far towards the finding of various new compounds with potential therapeutic value for the development of new chemotherapeutic agents. *Avicennia marina* has received some attention in determining its important chemical constituents. A naphthoforan compound with phytoalexin activity has been isolated **(Sutton *et al*., 1985; Miles *et al*., 1999).**

Sharaf and his co-workers (2000) reported that the *A. marina* possesses an in vitro cytotoxicity and anti-plasmodial activity. Lately, the chemotherapeutic activity of several naphthoquinones along with their analogues isolated from *Avicennia* plants was observed **(Itoigawa *et al.*, 2001).** The roots and bark of *A. marina* are recognised to include the tannin lapachol **(Tomlinson, 1994).** The fruits and leaves and of *Avicennia marina* are utilised in folk medicine for treatment of skin diseases. There is no information on the mosquito larvicidal or repellent activities of marine plants (mangroves and seagrasses) inhabiting the Red Sea. The current research is carried out to observe the mosquito larvicidal activity of Mangrove *Avicennia marina* of Yanbu, Saudi Arabia extracts against those of Culex pipiens, the main vector of lymphatic filariasis. This study focused on characterization and analysis the phytochemicals screening by Gas chromatography–mass spectrometry (GC-MS), that will exhibit additional clues about figuring out the formula of biomolecular therapy in drug studies.

MATRIALS AND METHODS

Collection and preparation of samples

Fresh leaves, stems, seeds and flowers of *Avicenna marina* was collected from Yanbu mangrove stand during November 2014. Mangrove leaves, stems, seeds and flowers of *Avicennia marina* were separately cleaned with seawater to get rid of epiphytes, shells and various extraneous matter and were instantly moved to separate polyethene bags and preserved in ice until resuming to the laboratory. Every varietywas repeatedly washed in running water and final once with distilled water. The plants were dried at room temperature (28±2˚C). The collected samples were packed in plastic bags and transported to the laboratory. These were washed with fresh water for removing sand, epiphytes and any extraneous matter; then dried in the shade for one week and powdered using a pistol and mortar.

Extraction of plant material

Extraction was carried out with ethyl acetate solvent at ambient temperature, following the method of **Abraham *et al.* (2015)** (Ethyl Acetate was used mainly to obtain medium hydrophobic compounds including medium and low polar neutral, basic and acidic compounds. The solvent was removed by rotary evaporator. The attained residue is used for *in vitro* screening of antimicrobial activity. The phytochemical GC-MS analysis of *Avicennia marina* plant extract was investigated.

Identification of the volatile constituents

The identification of volatile constituents of mangroves was made by application of Gas Chromatography equipped with Mass spectroscopy **(**GC/MS) hp HEWLETT PACKARD 5890 SERIES II. The prepared volatile constituents were analysed by gas chromatography (GC/MS) using the following conditions:

Injector temperature: 220˚C

Ion - source temperature: 280˚C

Colum/ Fused silica capillary Colum, (5% - phenyl Methylesiloxane) (DB-5)

Internal diameter: (25m x 0.025mm x 0.025mm).

Carrier gas: Helium gas (99.999 %) at flow rate of 1m/min

Oven temp program: 100-280ºC (increase of 2ºC/ min for 90 min)

Flow rate: 1.2 mL/min

Samples size: 1µL

Total run time: 120 min

Detector: Mass

Mass spectra and scan interval: 70 eV & 0.5 s

Mass fragmentation: 50- 550 Da

Identification of the volatile constituents was achieved through searching the database Willey 229LIB and comparing their mass fragmentation patterns with those of the available published data (Adams, 1989).

Antimosquitocidal activity

Tested insect

A colony of *Culex pipiens*was established in the laboratory, where mosquito larvae were firstly collected from small ponds. They were reared in enamel pans (30 cm, in diameter and 10 cm in height) and fed daily upon a mixture of dried powder bread, yeast and dried milk in the ratio of 2:1:1, respectively. Emerging adults were successively reared under room temperature (27 ± C º) in plastic cages of 30×30×30 cm, and fed on 10% sucrose solution which was offered in a piece of sponge stopped with a cable thread through the top of the cage. Larvae of the 3rd instar were mounted and identified. After inbreeding for several generations, five rafts were introduced into each enamel containing the nutrient solution mentioned above. Homogeneous larvae of thethird instar were isolated subsequently for running bioassay tests.

Tested procedure

The method of WHO (1975) was followed for testing mosquito larvicides. Pilot screening tests were carried out at a concentration level of 1000 ppm (w/v) to investigate the toxicity of the studied plant extracts. 0.5 mg of each tested crude extract was dissolved in 10 mL of solution and transferred into a 100mL glass beaker marked at 50 mL volume.

Twenty-five 3rd instar mosquito larvae of *Culex pipiens* were transferred to a beaker in the least quantities of water using a small dropper. Then the solution level was adjusted to a 50 mL. Five to seven concentrations were tested. Larval mortality was counted after 24, 48, 72 hours and exposed to log-probit regression analyses **(Unkelbach, 1985).** This process was repeated in three other beakers for each tested extract. Control experiments were carried out alongside other treatments where the same solution was used alone without plant extract, and then examined by calculating the percentage of mortalities:

$$\% \text{ corrected mortality} = \frac{\% \text{ test kill} - \% \text{ control kill} \times 100}{100 - \% \text{ control kill}}$$

Only promising extracts (i.e. of mortality equals to 50/or more) were subjected to detailed toxicity studies where their LC_{50} and LC_{90} values were determined. Thiswas accomplished according to the well-established methods (e.g. **WHO, 1975).** In this respect, a different range of concentrations of each concerned extract was preparedtoobtain mortalities ranging from 20% to 90%. At least four replicates were usually carried out for each tested concentration to prepare the LC-p line according to **Finney (1955).**

RESULTS AND DISCUSSION

Phytochemical Screening

The results of phytochemical screening of *Avicennia marina* leaves and their seeds extract in ethyl acetate showed the existence of flavonoids, sterols, alkaloids, saponins, carbohydrates, tannins coumarins, glycosides and terpenes (Table 1). *Avicennia marina* leaves showed a high content of flavonoids, the moderate content of saponins, sterols and or terpenes, glycosides and /or carbohydrates, low content of alkaloids, coumarins and tannins. The anthraquinones were totally absent. As for *Avicennia marina* seeds alkaloids, coumarins, flavonoids, saponins, sterols, tannins and glycosides and /or carbohydrates showed low content with the absence of anthraquinones.

Table (1): Preliminary phytochemical screening of *Avicennia marina* leaves and seeds.

Sl. No	Constituents	*A. marina* (leaves)	*A. marina* (seeds)
1	Alkaloids	+	+
2	Anthraquinones	-	-
3	Coumarins	+	+
4	Flavonoids	+ + +	+
5	Saponins	+ +	+
6	Sterols and Terpenes	+ +	+
7	Tannins	+	+
8	Glycosides and Carbohydrates	+ +	+

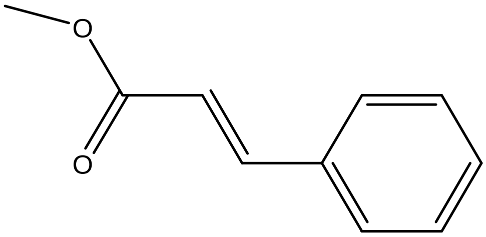
(+ + +) High content, (+ +) Moderate content, (+) Low content and (-) absent.

GC-MS Analysis

The results of GC-MS analysis of ethyl acetate extracts of *A. marina* leaves, seeds, flowers, stems are given in Table 2. In the GC-MS analysis of *A. marina,* the compounds found were a2-propenoic acid-3-phenyl ester, 3-acetyl methoxyphenyl, phenol, benzaldehyde,3-hydroxyl-4-methoxy, 1,2-benzenediol, phosphonic acid, *p*-hydroxyphenyl, 4H-pyran-4-one, 2,3- dihydro-3,5- dihydroxy-6-methyl and 4-hydroxy benzenesulfonic acid. The proposed chemical structures of these compounds are given in Figure 1.

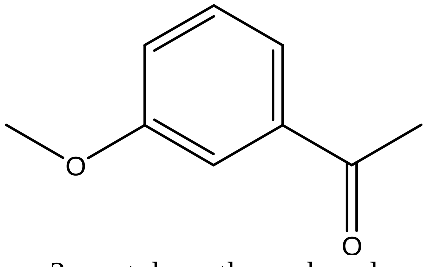
Table (2): Total ionic chromatogram (GC–MS) of ethyl acetate extract of mangrove plants obtained with 70 eV using a (DB-5) column (25 m x 0.025 mm x 0.025 mm) with He gas as the carrier.

Sl. No.	Plant parts	Compounds	Retention times (min)	Peak Area %	Mol. FW	Mol. Wt
1.	*Avicennia marina* leaves	2-Propenoic acid, 3-phenyl ester	5.459	93	$C_{10}H_{10}O_2$	162
2		3-Acetyl methoxyphenyl	4.426	80	$C_9H_{10}O_2$	150
3	*Avicennia marina* seeds	Phenol	4.526	72 (0.99)	C_6H_6O	94
4		Benzaldehyde,3-hydroxyl-4-methoxy	5.745	64	$C_8H_8O_3$	152
5		1 , 2 - Benzenediol	3.190	49	$C_6H_6O_2$	110
6	*Avicennia marina* flowers	Phosphonic acid, p-hydroxyphenyl	2.260	52	$C_6H_7O_4P$	174
7	*Avicennia marina* stems	4H- Pyran- 4- one, 2,3- dihydro-3,5-dihydroxy-6-methyl	2.952	78	$C_6H_8O_4$	144

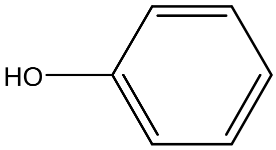


3-phenyl-2-propenoic acid methyl ester3-phenyl-acrylic acid methyl ester

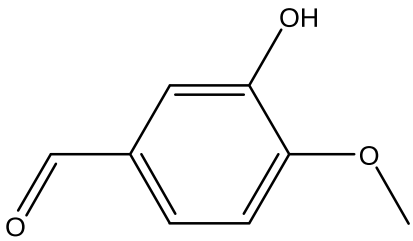
(1)



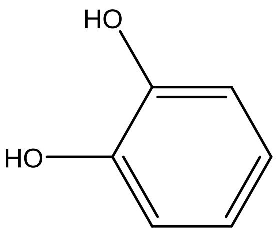
3- acetyl- methoxyphenyl
(2)



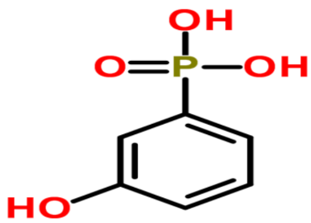
Phenol
(3)



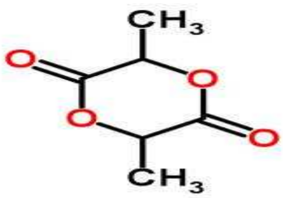
Benzaldehyde,3-hydroxyl-4-methoxy
(4)



1 , 2 – Benzenediol
(5)



Phosphonic acid, p- hydroxyphenyl
(6)



4H-pyran-4-one, 2, 3-dihydroxy 6methyl
(7)

Fig. (1): The structure of compounds identified by GC-MS according to table 2.

The previous literature has reported that *A. marina* contains tannin, a phenolic group, alkaloids, xanthoproteins, resins and coumarin **(Jia *et al.*, 2004).** Several steroids and terpenoids are found in the leaves and flowers of *A. Marina,* which were identified by GC-MS technique. **(Jia *et al.*, 2004).** The results of photochemical screening of *Avicennia marina* leaves and their seeds and *Rhizophora macronata* leaves contains alkaloids, coumarins, flavonoids, saponins, sterols and terpenes, tannins, glycosides and carbohydrates. The presence of flavonoids has important effects on plant biochemistry and physiology as antioxidants, an enzyme inhibitor, precursors of toxic substances and these are also recognised to possess anti-inflammatory, antioxidant, anti-allergic and anti-carcinogenic activities. The present results agreed with those of literature **(Prabhu and Guruvayoorappan, 2012).**

In this study, the leaves extract of *A .marina* resulted in the isolation of compounds namely 2-propenoic acid, 3-phenyl ester, 3-acetyl methoxyphenyl and phenol. In the same way, the ethyl acetate extract from the seeds of *Avicennia marina* resulted in the isolation of 2 compounds namely benzaldehyde, 3-hydroxyl-4-methoxy and 1,2-benzenediol. GC-MS of *Avicennia marina* flowers led to the isolation of only two compounds *viz.* phosphonic acid, p-hydroxyphenyl (Fig. 2). Benzenesulfonic acid, 4-hydroxy was found in both *Avicennia marina* flowers and *R. mucronata*. *Avicennia marina* seeds. *R. mucronata* has compounds which have the same chemical structures and the molecular weight but have different retention time and mass fragmentation due to the presence of the compounds namely 1,2-benzenediol and 1,4-bBenzenediol isolated by extracted from *A. marina* seeds and *R. Mucronata*, respectively.

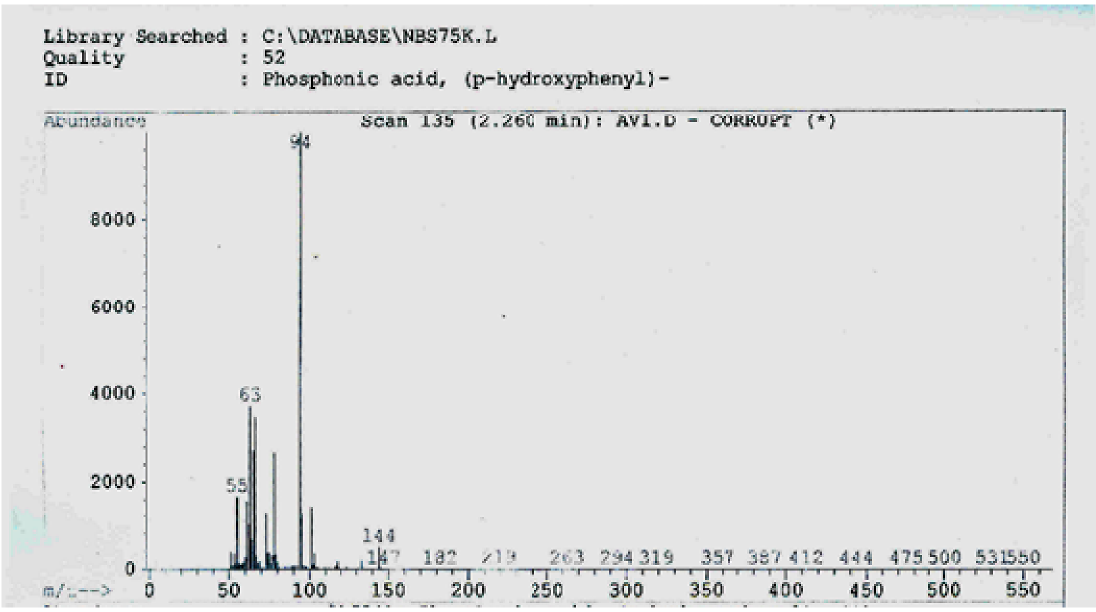


Fig. (2): The mass spectrum of Phosphonic acid, p- hydroxyphenyl

Culex pipiens is the most widely distributed mosquito species in the world. **Hoogstraal *et al.* (1977**) stated that mosquitoes in Egypt are vectors of malaria, various forms of filariasis and numerous arboviruses like dengue and yellow fevers. **Thangum and Kathiresan (1996)** in Parangipettai studied a large number of marine plants as insecticidal and /or repellent activities against mosquito in India. Their study was the first to investigate the smoke repellent activities of seaweeds, seagrasses and mangrove plants for their larvicidal against several mosquito species. Study of environmental hazards in using synthetic insecticides against mosquito was also conducted by **Bahgat *et al.* (2001)** by using spinosad, which is produced from soil Actinomycete. Our results showed that extracts of seeds and leaves of *Avicenniamarina* were more effective than other parts of the same mangrove plant as well as of the mangrove *R. mucronata*. The results also displayed that some extracts of *H. stipulacea* are more susceptible against *Culex pipiens* larvae.

There have been numerous studies on the mosquito larvicidal activity of terrestrial plants **(Kathiresan and Thangam, 1987).** Subsequently, the mosquito larvicidal activity of the seaweeds *Plocamium telfairiae* and *Laurencia nipponica* was reported by **Watanabe *et al.* (1989 and 1990)** who isolated mosquito larvicidal compounds. Ours was the first study on the mosquito larvicidal activity of Saudi marine plants. Current repellent compounds, like dimethyl phthalate which are available in the market, are expensive and provide the protection for a short period **(Kalyanasundaram *et al.*, 1986).**

In view of these results, the purified active compounds from the most effective samples found in our studies could be efficient at getting rid of mosquito larvae or repelling adult female mosquitoes within an economical and secure way. The next conclusion could be beneficial in the field of mosquito management without harming the environment.

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ARABIC SUMMARY

المكونات الكيميائية والأنشطة الطاردة للبعوض لمستخلصات نبات المانغروف أفيسينيا مارينا من ينبع، المملكة العربية السعودية

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أشجار المنغروف هي أشجار وشجيرات تنمو على سواحل البحار المالحة في المناطق المدارية والمناطق شبه الاستوائية. وفي الوقت الحالي، يشار إلى المنغروف كمورد ثمين للمكونات الكيميائية ذات قيمة علاجية وزراعية محتملة. ومع ذلك لم يتم دراسة المكونات الكيميائية للعديد من نباتات المنغروف على نطاق واسع، وكشفت الدراسات حاليا عن العثور على مركبات متنوعة ومختلفة ذو قيمة علاجية محتمل استخدامها لتطوير العلاج الكيميائي الحديث.

ونظرا لعدم توافر معلومات عن أنشطة مكافحة أو طرد البعوض باستخدام مستخلصات من النباتات البحرية (المنغروف والأعشاب البحرية) التي تعيش في البحر الأحمر.لذلك تم إجراء البحث الحالي لمراقبة النشاط البيولوجى المقاوم للبعوض من المنغروف أفيسينيا مارينا ينبع، المملكة العربية السعودية .

تم التحليل الكيميائي النباتي بواسطة كروماتوجرافيا الغاز ذوالطيف الكتلى. لكل من بذور وزهور وسيقان منغروف أفيسينيا مارينا . تشير النتائج إلى وجود، االأستيرودات، والتنينات، جليكوسيدات، الكربوهيدرات، الصابونين، ستيرول، تيربينويد والفينولات. كشفت التحاليل وجود المكونات الطبية النشطة وساعدت هذه الدراسة أيضا على تحديد صيغة وهيكل العلاج الجزيئي الحيوي التي يمكن استخدامها كأدوية. تم اختبار أفيسينيا مارينا للأنشطة المقاومة للبعوض. تم فحص السمية المختبرية ضد. سلالة من يرقات الطور الثالث إنستار من كيولكس بيبينز. وأظهرت النتائج أن مستخلصات بذور وأوراق أفيسينيا مارينا كانت أكثر كفاءة من الأجزاء الأخرى ضد يرقات الطور الثالث من كيولكس بيبينز البعوض. في ضوء هذه النتائج، يمكن أن تستخدم المركبات النشطة المفصولة من العينات الأكثر فعالية في قتل يرقات البعوض أو ضد بعوض الإناث البالغ بطريقة اقتصادية وآمنة. ويمكن أن يكون الاستنتاج الحالى مفيدا في مجال مكافحة البعوض دون الإضرار بالبيئة.