

Phytochemical Analysis of *Avicennia marina* and *Rhizophora mucronata* by GC-MS

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ABSTRACT

In this study, the phytochemical analysis of *Avicennia marina* and *Rhizophora mucronata* have been studied. *Avicennia marina* leaves, seeds, flowers, stems and *Rhizophora mucronata* leaves indicate the presence of, Steroids, Tannins, Glycosides, Carbohydrates Saponnins, Sterols, Terpenoids and Phenol. In the GC-MS analysis, ten bioactive photochemical compounds were identified in the ethyl acetate extract of *Avicennia marina* leaves, seeds, flowers, stems and *Rhizophora mucronata* leaves. The *Avicennia marina* and *Rhizophora mucronata* revealed the presence of medicinal active constituents by GC-MS. This study also helped to identify the formula and structure of bimolecular therapy which can be used as drugs.

Keywords: Phytochemical screening of *Avicennia marina* and *Rhizophora mucronata* by GC-MS.

INTRODUCTION

Mangroves are trees and shrubs that grow in saline coastal habitats in the tropics and subtropics-mainly between latitudes 25° N and 25° S (Saenger, 2002). They have recognized 65 mangrove species in 22 genera and 16 families (Kathiresan and Bingham, 2001).

Recent studies have shown that the plant extract has a remarkable hepatoprotective effect (Miles *et al.* 1998). Recently, it has been strongly recommended that mangroves should be considered as a valuable source for chemical constituents with potential medicinal and agricultural values (Miles *et al.* 1998). Although the chemical constituents of most mangrove plants still have not been studied extensively, investigations have led so far to the discovery of several novel compounds with prospective medicinal value for the discovery of new chemotherapeutic agents.

Avicennia marina (Forssk.) Vierh. (*Avicenniaceae*) has received some attention in determining its important chemical constituents. A naphthofuran compound with phytoalexin activity has been isolated (Sutton *et al.* 1985; Miles *et al.* 1998). Fatty acids, sterols and hydrocarbons had been studied in relation to their chemotaxonomic significance in eleven mangrove species including *A. marina* (Hogg and Gillan 1984).

The presence or absence of an iridoid glucoside 2-cinnamoyl mussaenosidic acid from *A. marina* extracts can be used in subspecific chemotaxonomy (Bousquet-Mélou and Fauvel 1998). The present study focused on characterization and analysis the phytochemicals screening by GC-MS, which will throw more insight into identifying the formula of bimolecular therapy in drug studies. *Avicennia marina* and *Rhizophora mucronata* are a commonly available as mangrove plant in almost all the coastal Red Sea of Egypt.

It is a folklore medicinal plant used mainly against rheumatism, paralysis, asthma and snake-bites, skin disease and ulcer. Indian mangrove is a folk remedy for

boils and tumors. A resinous substance excluded from the bark acts as a contraceptive and apparently can be taken all year around without ill effects (Thirunavukkarasu *et al.*, 2010).

MATERIALS AND METHODS

Collection and preparation of samples

Fresh leaves of *Avicenna marina* and *Rhizophora mucronata* were collected from Gharqana coast. Nabq protected area and Ras Mohammed national park at southern Sinai coast (Gulf of Aqaba) during November 2007.

Mangrove leaves, stems, seeds and flowers of *Avicennia marina* and whole plant of *Rhizophora mucronata* were separately cleaned with many changes of seawater in order to remove epiphytes, shells and other extragenous matter and were immediately transferred to separate polythene bags and placed on ice till return to the laboratory.

Each species was again cleaned in running tap water and further once with distilled water and shade dried under room temperature (28±2°C) for further use. The collected samples were packed in plastic bags and transported to the laboratory.

They were washed with fresh water for removing sand, epiphytes and any extraneous matter; then dried in shade for five to seven days and powdered using a pistol and mortar.

Extraction of plant material

Extraction was carried out with ethyl acetate solvent at ambient temperature. The solvent was removed under reduced pressure; the residue obtained was finally dried under vacuum and used for in vitro screening of antimicrobial activity.T

he phytochemical, GC-MS and analysis of *Avicennia marina* and *Rhizophora mucronata* plant extract was investigated.

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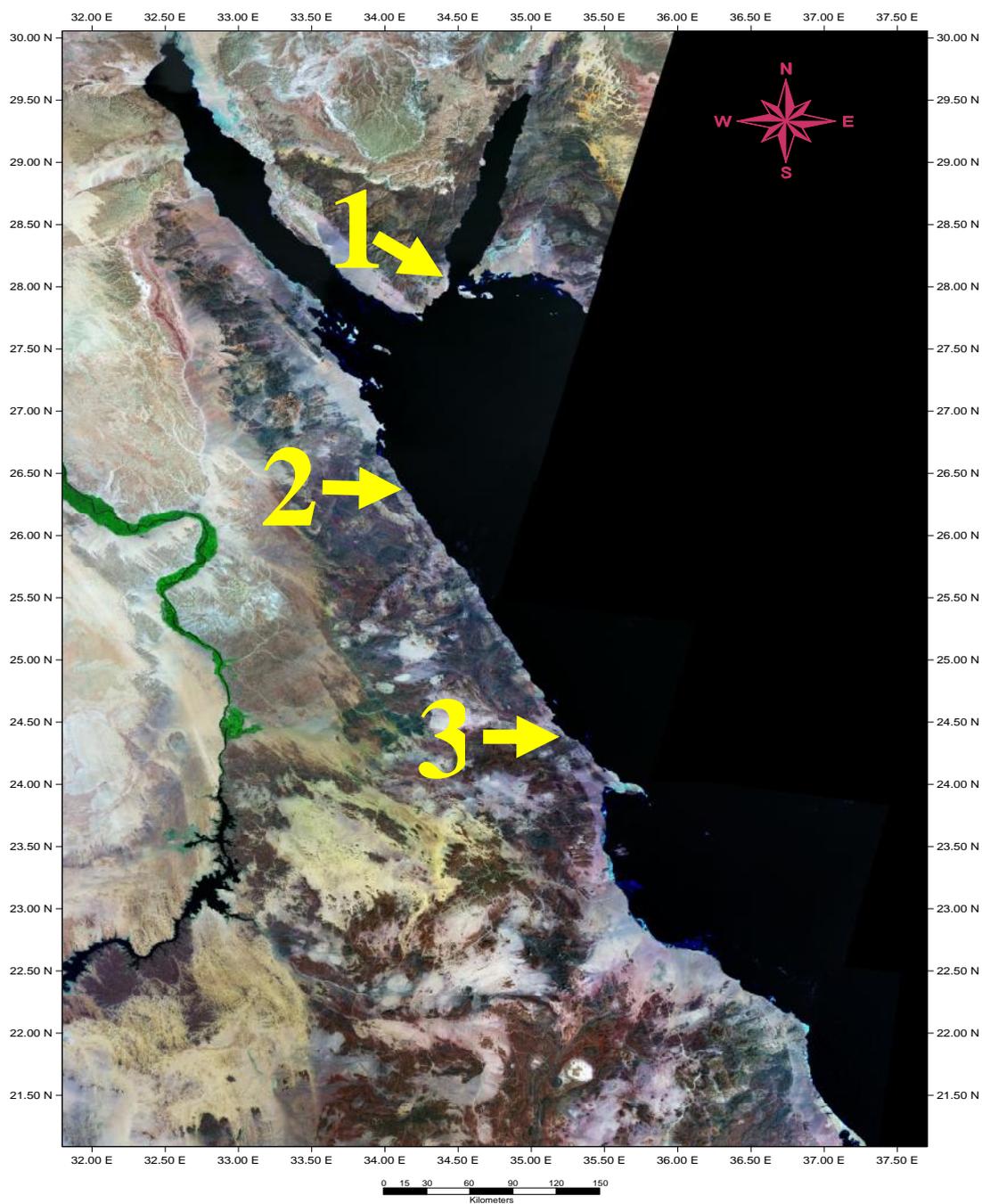


Figure (1): Satellite image showing the Egyptian Red Sea Coasts. Arrows indicating the study sites. 1- Safaga area, 2- Nabq protection, 3- Wadi El Gemal Island.

Identification of the volatile constituents

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

Initial temp: 120°-300° rate 2°-min. for 90 min
 Total run time: 120 min
 Flow rate: 1.2ml/min
 Colum/ Fused silica capillary Colum, (5% - phenyl Methylsiloxane) (DB-5)
 Internal diameter: (25m x 0.025mm x 0.025mm).
 Samples size: 1µl
 Carrier gas: Helium gas

Injection temperature: 220°C/min

Identification of the volatile constituents was achieved by library searched data base Willey 229LIB and comparing their mass fragmentation patterns with those of the available published data (Adams, 1989).

RESULTS

Phytochemical Screening

The results of phytochemical screening of *Avicennia marina* leaves and their seeds and *Rhizophora macronata* leaves revealed the presence of alkaloids, coumarins, flavonoids, saponins, sterols and terpenes, tannins, glycosides and carbohydrates (Table 1).

Avicennia marina leaves showed high content of flavonoids, moderate content of saponins, sterols and or terpenes, glycosides and /or carbohydrates, low content of alkaloids, coumarins and tannins. While anthraquinones were totally absent. As for *Avicennia marina* seeds alkaloids, coumarins, flavonoids, saponins, sterols, tannins and glycosides and /or carbohydrates showed low content with absence of anthraquinones. *Rhizophora macronata* leaves had moderate content of flavonoids, saponins, sterols and or terpenes and coumarins. Also the *R. mucronata* leaves showed low content of alkaloids, sterols, tannins, and carbohydrate.

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

Constituents	<i>A.marina</i> (leaves)	<i>A. marina</i> (seeds)	<i>R. macronata</i> (leaves)
1-Alkaloids	+	+	+
2-Antraquinones	-	-	-
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 and *R. mucronata* leaves are given in Table 2. In the GC-MS analysis of *A. marina*, the 2- propenoic acid, 3-phenyl ester was found to be a major constituent with a peak area of 93.00 % and retention time 5.46, followed by Ethanone, 1-3-methoxyphenyl with a peak area of 80 % and retention time 4.43 and 4H- Pyran- 4- one, 2, 3- dihydro-3, 5- dihydroxy-6-methyl with a peak area of 78

% and 2.95 retention time, respectively. In GC-MS analysis of *R. mucronata* leaves reveals that 2- Furancarboxaldehyde, 5-hydroxymethyl was found to be the major constituent with peak area of 91% and retention time of 3.48, followed by 1, 4 – Benzenediol with a peak area of 53% with retention time of 3.86 and Benzenesulfonic acid, 4-hydroxy with a peak area of 40% and retention time of 2.26, respectively.

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

No	Sample	Compound	R.t.min	%	M.F	M.wt
1	<i>Avicennia marina</i> leaves	2- propenoic acid, 3-phenyl ester	5.459	93	C ₁₀ H ₁₀ O ₂	162
		3-acetyl methoxyphenyl	4.426	80	C ₉ H ₁₀ O ₂	150
		Phenol	4.426	72	C ₆ H ₆ O	94
2	<i>Avicennia marina</i> seeds	Benzaldehyde,3-hydroxyl-4- methoxy	5.745	64	C ₈ H ₈ O ₃	152
		1, 2 - Benzenediol	3.190	49	C ₆ H ₆ O ₂	110
3	<i>Avicennia marina</i> flowers	Phosphonic acid , p-hydroxyphenyl	2.260	52	C ₆ H ₇ O ₄ P	174
4	<i>Avicennia marina</i> stems	4H- Pyran- 4- one, 2,3- dihydro-3,5- dihydroxy-6-methyl	2.952	78	C ₆ H ₈ O ₄	144
5	<i>Rhizophora</i> <i>mucronata</i> leaves	1, 4 - Benzenediol	3.856	53	C ₆ H ₆ O ₂	110
		2-Furancarboxaldehyde,5- hydroxymethyl	3.476	91	C ₆ H ₆ O ₃	126
		4-hydroxy Benzenesulfonic acid	2.260	40	C ₆ H ₆ O ₄ S	174

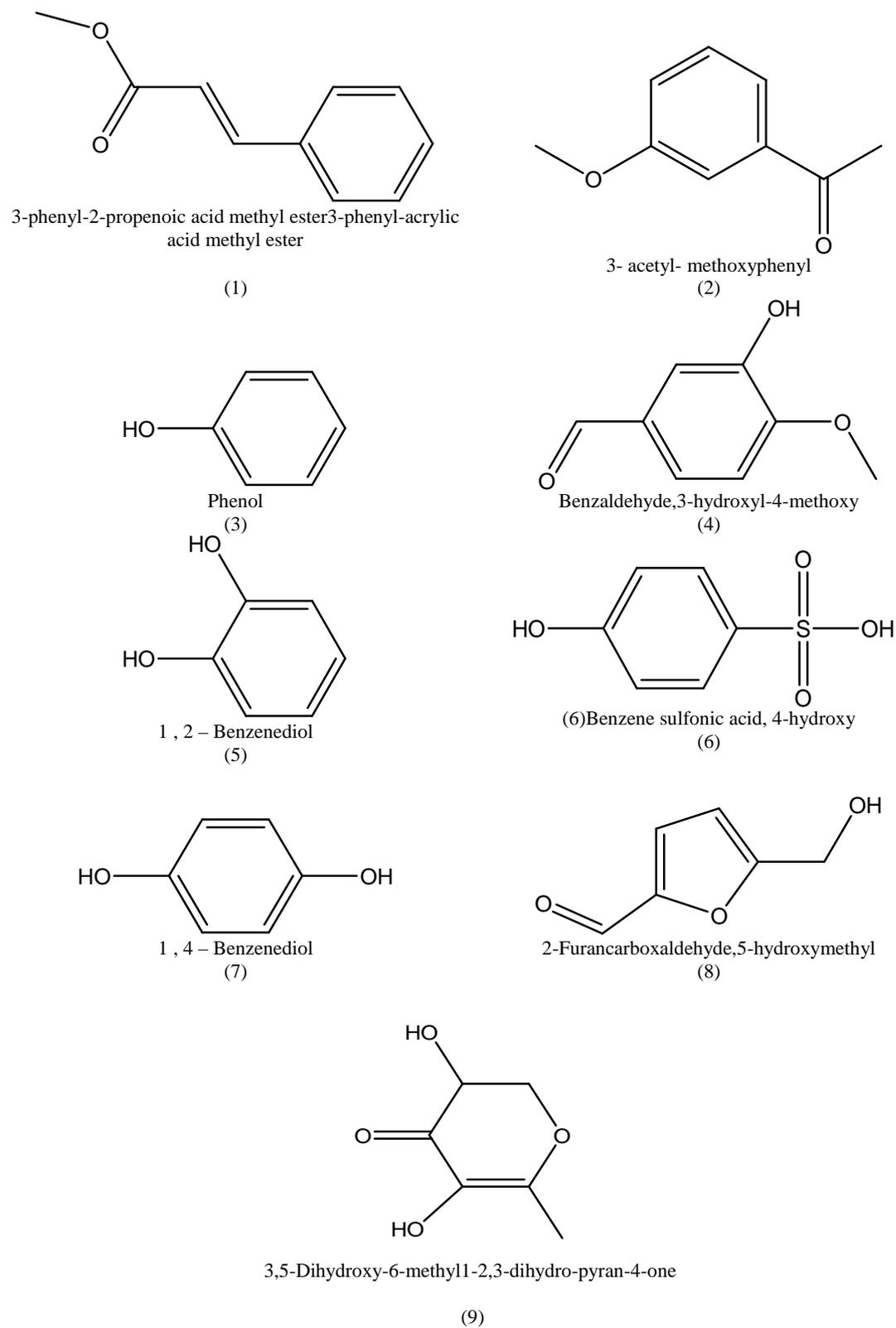


Figure (1): Showed the structure of compounds identified by GC-MS according to table 2.

DISCUSSION

Previous literature has reported that *A. marina* contains tannin, phenolic group, alkaloids, xanthoproteins, resins and coumarin (Jia *et al.*, 2004) and many terpenoids and steroids exist in the barks leaves, flowers of *A. marina* 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, coumarine, flavonoids, saponins, sterols and terpenes, tannins, glycosides and carbohydrates. The presence of flavonoids has important effects on plant biochemistry and physiology as antioxidants, enzyme inhibitor, precursors of toxic substances and they are also recognized to possess anti-inflammatory, antioxidant, anti-allergic and anticarcinogenic activities. Our results agree with those of retrature (Prabhu V. V and Guruvayoorappan, C. 2012).

In this study, the ethyl acetate extract of the leaves 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 extracted from the seeds of *Avicennia omarina* resulted in the isolation of 2 compounds namely Benzaldehyde, 3-hydroxyl-4-methoxy and 1, 2 - Benzenediol . GC-MC of *Avicennia marina* flowers resulted in the isolation of only one compound namely Phosphonic acid , p-hydroxyphenyl. Benzenesulfonic acid, 4-hydroxy was found in both *Avicennia marina* flowers and *R. mucronata*. *Avicennia marina* seeds and *R. mucronata* have compounds which have the same chemical structure 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 – Benzenediol isolated by extracted from *A. marina* seeds and *R. mucronata* respectively.

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مسح كيميائي للنباتات البحرية (أشجار الشورى) لافيسنيامارينا والريزوفورامكروناتا باستخدام جهاز كروماتوجرافيا الغاز – الكتلة

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الملخص العربي

تم تجميع عينات لبعض النباتات البحرية (*Avicennia marina* and *Rhizophora mucronata*) عمل مسح كيميائي للنباتات المذكورة انفا باستخدام جهاز كروماتوجرافيا – الكتلة وخلال هذه الدراسة لمستخلصات الخام لنباتات المنجروف وأسفرت نتائج هذه الدراسة عن وجود زيوت طيارة فلا فونيدات فى النباتات واستيروولات تانينات وصابونيات تربينات و كومارينات وتحليل الزيوت الطيارة للنباتات الموجودة للنباتات المستخلصة باستخدام جهاز كروماتوجرافيا الغاز – . وجد انها تحتوى على عشرة مركبات على

:
- فيسنيامارينا وهم :

- - بروبانويك اسيد فينايل إستر .

- - اسيتيل ثلاثه ميثوكسي فينيل .

- فينول .

- مركبان ليذور افيسنيامارينا وهم :

- بنزيلدهايد - هيدروكسي ميثوكسي .

- , - بنزيندايول

- مركب واحد للازهار افسينيا مارينا فوسفونيك اسيد باراهيدروكسي فينايل .

- ثلاثه مركبات لاوراق الريزوفورامكروناتا وهم :

- , - بنزين دايلول

- فيوران كاربوكسا الدهايد هيدروكسي ميثايل

- بنزين سلفونيك اسيد - هيدروكسي

- مركب واحد للساق افيسينا مارينا وهو , داي هيدروكسي - ميثايل , داي هايدروكسي

بايران , .

وإجمالاً لنتائج هذا البحث ، فقد وجد ان هناك علاقة وطيدة بين استخدامات نباتات المنجروف (افيسنيامارينا وريزوفورامكروناتا) فى الطب الشعبى للنباتات المختلفة حيث يعد من النباتات الواعدة للدراسات المستقبلية الكيميائية والصيدلانية .