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# The Phytochemical Analysis and Antimicrobial Activity of *Pergulariato mentosa* in North East Kingdome of Saudi Arabia KSA

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**The increment in numbers of antimicrobial resistant strains along with the scarcityof new targets for drugindustryhas forcedscientists to investigate deeply in the natural resources for new compounds with antimicrobial activity. *Pergularia tomentosa* is a member of the Apocynaceae family found in a wide geographical region including the Gulf region, Africa,andthe Middle East. It is used as a remedy for the treatment of skin sores, asthma,and bronchitis. Dried plants of *Pergularia tomentosa* were subjected to extraction by using a Soxhlet extractor process to obtain essential oil and characterized by HPLC- Mass Spectroscopy (GC-MS). The essential oil was evaluated forantibacterial activity against pathogenic microorganisms by well diffusion method and confirmed by microdilution method. Additionally, we measured the antioxidant activity of the extracts using DPPH reagent. Phytochemical analysis has revealed variation in compositions and concentrations of *P. tomentosa* constituents grown in Hail from otheragricultural regions. The lowest MIC was recorded withethylacetate extract MIC of 6.25 mg/ml against *S. typhi,* whereas, the ethanolic extract had the broadest effective against the five strains with a MIC of 25 mg/ml. In conclusion, we summarize a variation between the phytochemical constituents of *P. tomentosa*plants grown in the district of Hail and other geographical regions. In addition, there are several natural phytocompounds with an antimicrobial activity could be a good target for theantimicrobial and antioxidants industry.**

**Keywords:** *Pergularia tomentosa*, Antimicrobial, Phytochemical, Antioxidants, HPLC-MS.

The emergence of superbug strains between health care workers has obliged the scientific society to investigate deeper in the natural products for alternative compoundswithantimicrobial

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activity[1, 2]. According to a report by the Infectious Diseases Society of America, around 70% of new cases administered to the hospitals in the US are involved with strains that show a potential no susceptibility to at least one drug [3, 4]. In the UK, nowadays, methicillin-resistant Staphylococcus aureus (MRSA) is considered a real concern in infection control accounting for around more than 50% of all *S. aureus* isolates whereas it had a low value a decade ago[2].

Since the beginning of mankind, there is a wide use of herbal plants in the folk medicine due to their therapeutic and pharmaceutical properties[5-8].*Pergularia tomentosa* is a member of the Apocynaceae family (subfamily: Asclepiadeae) a yearly green plant with a distinctive odor,known in Hail city as "Aloonah"[9, 10]. The plant is a climbing to semi erect perennial herb of around 30 cm, the stem is pale green-white in color, highly branched and usually grow vertically and milky latex is extracted from the plant [11].

This plant is found in a widegeographical regionincluding Gulf region (Saudi Arabia and Oman),Africa (north Sudan, Egypt, Ethiopia, Algeria, Niger and Kenya) and Middle East (Jordan, Iraq, Iran, Pakistan, and Afghanistan,)[5, 12-14]. *Pergularia tomentosa*is used as a remedy for the treatment of rheumatic fever, asthma, bronchitis, helminthiases and skin sores as cutaneous leishmaniosis[15, 16]. Furthermore, several publications havereportedthe cytotoxic, antioxidant and antibacterial activityof *P. tomentosa*[17-19]. Up to date, this is the first study conducted on *P. tomentosa* in the region of Hail north east KSA that is focusing on the analysis ofphytochemical components and elucidating theantioxidant and antimicrobial activityof this perennial.

**Fig. 1.** *Pergularia tomentosa*

## MATERIALS AND METHODS

### Sampling

This study was performed in the northeast region of KSA at Hail district. *Pergularia tomentosa*(Fig. 1) was collected from the surrounding regions ofNaqbeenvillage (a mountainous area) about 25 km from Hail.

Leaves stem,and roots of *P. tomentosa*were collected locally in March 2017 in Hail, Southeast of KSA. Samples were collected from the waterways and slope in hilly areas and mixed together. The authenticity of the plant was confirmed by Dr. Mohammed Ahmed, and voucher specimens are maintained at the University of Hail (UOH) herbarium.

### Preparation of plant extracts

The dust-freeleaves stem and roots of *Pergularia tomentosa*were shade dried for five daysand finely grounded. 30grams of dried powder were mixedin equal amounts from each part and extractedwith 200 ml of ethanol, chloroform,andethylacetate, consecutively,using Soxhlet extractor in order toseparatephytochemical compounds based on their polarities. Filter paperWhatman No.1 was used to filter the crude extracts to remove impurities and debris and then the yield was concentrated by applying a vacuum at 35°C using a rotary evaporator. The concentrated extracts were subsequently dried aseptically using Lyophilization. Millipore distilled water was used to constitute inappropriate volume to obtain a final concentration of 200 mg/ml solution.

### Phytochemical studies

For choosing the best solvent system (gradient of solvents (A and B)), we setup the method according to the TLC and analytical HPLC/ UV. The concentration of the extract for the LC-MS analysis was 1 mg/2 ML [20].

Ethanol layer of the extract of *P. tomentose* was subjected to qualitative analysis to determine the phytochemical composition for the plant. Liquid Chromatography-Mass Spectrometry (UHPLC system) with an autosampler and Waters nano Acquity HSS T3, 1.8 ìm, 100 ìm × 100 mm column was used in the analysis. $H_2O$ 0.1 % formic acid (A) (v/v, pH=2.17) and 90 % acetonitrile in

$H_2O$0.1 % formic acid (B) was used asmobile phases at a flow rate of 0.4 ml/min. 1ìm injection volume was used, the gradient elution for the injection was 5 % B during 0–2.5 min, a linear increase from 5 to 25 % B during 2.5–20 min, from 25 to 40 % B during 20–40 min and from 40 to 50 % B during 40–50 min, finally from 50 to 95 % B during 50–65 min followed by 15 min of maintenance. For identification of the eluent, we used Thermo Electron LTQ-Orbitrap XL mass spectrometer equipped with a nanoelectrospray ion source (ThermoFisher Scientific, Bremen, Germany) and operated under Xcalibur 2.1 version software, in positive ionization mode for the MS analysis using data-dependent automatic switching between MS and MS/MS acquisition modes (Table 1)[12].

**Determination of antioxidant activity**

Antioxidant propertiesof *P. tomentosa* extracts wasdetermined in terms of scavenging free radical using DPPH method[21-23].

The reduction in the concentration of 2,2-diphenyl-1-Picrylhydrazyl (DPPH) radicals by the activityof the antioxidantsin *P. tomentosa*was measured and quantified bya colorimetric method. 0.02 g of DPPH reagent was dissolved in 1 L methanol;3.9 mL of the solution is added to 0.1 mL of each sample that was diluted in a pure solvent of extraction at different concentrations. The mixtures were incubated for 90 minutes in a dark place at room temperature and the absorbance for each sample was measured at 517 nm using a UV/VIS spectrophotometer in triplicates.

The activity of free radical scavenging was disclosed as $IC_{50}$ (µg/mL). The following equation was used to calculate the reduction in the amount of freeDPPH radical by the extract:

DPPH scavenging effect (%) = ((A0-A1)/A0)*100

A0 is the control absorbance at 90 min, and A1 is the sample absorbance at 90 min. All samples were analyzed in triplicate.

**Antimicrobial activity**

We have conducted a preliminary study using the anti-bacterial susceptibility test to determine the minimal inhibitory concentrations (MICs) for thethree herbal extracts (ethylacetate, ethanol,and chloroform) against gram-positive, gram-negativeand fungalstrains. The protocol was conductedas defined in guidelines ofNational Committee for Clinical Laboratory Standard (NCCLS) [24]. Further, we compared their activities to reference compounds, fluconazole,andciprofloxacin. We have determinedMICs for a strain collection consisting of*Staphylococcus aureus*ATCC®35556,*Staphylococcus epidermidis* ATCC®12228,*Escherichia coli* ATCC® 25404,*Salmonella typhi*ATCC® 700931and *Candida albicans*SC5314. We covered a range of 6.25-100mg/mL for the herbal extract against the strains. Bacterial strains were propagatedon nutrient plates at37°C for 24 hours. Whereas, we propagated *C.albicans* strains on sabouraud dextrose (Sab) plates at 25 °C for 24 hours. Rownumber 1 on each microdilution platewas used as viability control ofmicrobial cells. Rows 2

**Table 1.** Chromatographic conditions of the HPLC MS

| HPLC Conditions | Pump Flow Rate | | Auto-sampler Injection Volume | | Auto-sampler Temperature | | Column Oven | |
|---|---|---|---|---|---|---|---|---|
| | 1.00mL/minute | | 25.00µL | | 10.0 ‟C | | 30.0 ‟C | |
| Chromatography | Mobile Phase | | Mixture of(60%ACN, +40%(675µL Triethylamine / 1L of mixture,),pH adjusted to 7.1with phosphoric acid | | | | | |
| | Column Type | | Sepax GP-C18, (150 × 4.6 mm, 5 µm) | | | | | |
| | Expected Retention Time | | As seen in Table 230 nm | | | | As seen in Table230 nm | |
| MRM-Detection Conditions | Anlytes | Q1 | Q3 | Dwell | FP | DP | EP | CE | CXP |
| | Category1 | 86 | 68.5 | 150 | 75 | 80 | 10 | 20 | 25 |
| | Category2 | 440 | 265 | 150 | 75 | 80 | 10 | 20 | 25 |
| Mass-Spectra Conditions | CUR | CAD | | IS | | TEM | | NEB | |
| | 10 | 6 | | 5500 | | 400 | | 5 | |

to 12 contained a decreasingamount of the herbal extract in a 1:1serial dilution schemeresulting in a range of final concentrations from 6.5mg/mL to 100 mg/mL. Ciprofloxacinwas used as areference drug for the antibacterial activity of the extracts whereas Fluconazole was used for the comparison ofantifungal activity. TECAN Microtiter plate reader was used for obtaining the optical density at 450nm forthe microtiter plates after incubation at37°C for 22 ± 2 hours, results were analyzedwith Magellan software.

## RESULTS

### Phytochemical compounds

Characterization of the ethanol fraction of *P. tomentosa* was carried out using mass spectrometry. We identified 18 compounds in theaqueous extract;14 compoundsbelonged to phenols and flavonoids whereas the last four had cardenolides structures. The results of the identified compounds were shown in Table 3. We also identified 3 flavonoids (Quercetin 3*O*-galactoside (8), Kaempferol 3-*O*-glucoside (11) and Kaempferol 3-*O*-malonylhexoside (14)) and one cardenolides (17) with LCMS techniques. This had also been isolated before in *P. tomentosa* [ref] and three other cardenolides (5, 17 and 18) which were not reported in this plant. Most of the identified flavonoids were belong to the flavone and flavonol [25, 26](Table 2, Fig. 2).

### The DPPH free radical scavenging activity

The property of *P. tomentosa* extracts toscavenge free radicals was measured using a UV-visiblespectrophotometer. Samples with plant extractswere analyzed covering a concentration range of 0.10 2.0 mg/mL. In the same conditions, Butylhydroxytoluene(BHT)was also measured as a reference compound for the radical scavenging activity.The proportion of DPPH free radicals scavengingfor both tested extracts and the positive control BHTare depicted inTable 3. The antioxidants properties of the extracts wereexpressed by the $IC_{50}$ values, showing the sample concentration required to reduce fifty percent of DPPH free radicals (Table 3).

Linear regression analysis of the dose-response curve was used to determine the sample concentration required to reduce DPPH radical by 50% ($IC_{50}$ value). The values are the mean of three determinations ± standard error.

### Antimicrobial activity

The three fractions of *P. tomentosa* showed different antimicrobial activity against

**Table 2.** Phytochemical components identified in the ethanolic extract of *P. tomentosa*

| | Identified Compounds in negative ion mode | Molecular formula | Antimicrobial activity | RT (min) | References |
|---|---|---|---|---|---|
| 1 | Phenolic glycosid: Scrophenoside D | $C_{28}H_{34}O_{17}$ | NO | 4.12 | Li et al, 2014 |
| 2 | Phenolic: Synapoyl hexoside | $C_{17}H_{22}O_{10}$ | NO | 4.7 | Orquedaet al, 2017 |
| 3 | Flavone: Luteolin-di C-lucoside | $C_{27}H_{30}O_{16}$ | YES | 5.12 | Singh et al, 2015 |
| 4 | Phenolic: Feruloyl Glucoside | $C_{16}H_{20}O_{9}$ | NO | 9.1 | Frig et al, 2016 |
| 5 | Cardenolide: antiaroside G | $C_{29}H_{42}O_{12}$ | NO | 10.6 | Shi et al, 2013 |
| 6 | Flavonole: Kaempferol-malonyl-dihexosid | $C_{30}H_{32}O_{19}$ | YES | 12 | Salced et al, 2016 |
| 7 | Flavonole: Kampferol + glucose | $C_{27}H_{28}O_{17}$ | YES | 12.5 | Heneidak et al, 2006 |
| 8 | Flavonole: Quercetin 3-0-galactoside | $C_{21}H_{20}O_{12}$ | YES | 13.5 | Valente et al, 2016 |
| 9 | Flavonole: Isorhamnetin-3-0-glucoside | $C_{22}H_{22}O_{12}$ | NO | 13.6 | Haijuan et al, 2013 |
| 10 | Flavonole: Kaempferol 3-0-glucoside | $C_{21}H_{20}O_{11}$ | YES | 13.9 | Hettwer, 2016 |
| 11 | Phenolic: Ferulylmalic acid | $C_{14}H_{14}O_{8}$ | NO | 14.4 | Song et al, 2016 |
| 12 | Phenolic: glucuronic acid | $C_{17}H_{14}O_{10}$ | NO | 14.5 | Heneidak et al, 2006 |
| 13 | Flavonole: Kaempferol 3-0-malonylhexoside | $C_{24}H_{22}O_{14}$ | NO | 15.3 | Dugo et al, 2009 |
| 14 | Flavonole: Kaempferol-3-0-6 -acetyl-b-Dglucopyranoside | $C_{23}H_{22}O_{12}$ | YES | 15.7 | Ojwang, 2012 |
| 15 | Flavonole: quercetin-3-(6"-succinoyl)-glucoside | $C_{25}H_{24}O_{15}$ | YES | 16.3 | Hamed et al, 2006; |
| 16 | Cardenolide: Hydroxycalactin | $C_{29}H_{40}O_{10}$ | NO | 16.9 | Piacente et al, 2009 |
| 17 | Cardenolide: Antiaroside E | $C_{29}H_{42}O_{10}$ | NO | 18 | Shi et al, 2010 |
| 18 | Cardenolide: Antiaroside F | $C_{35}H_{52}O_{15}$ | NO | 18.5 | Shi et al, 2010 |

the study strains; ethanol layer showed a broad inhibition activity against the five strains (*S. aureus, S. epidermidis, E. coli, S. typhi and C. albicans*) whereas ethyl acetate fraction had the most effective MIC values against only three strains (*S. epidermidis, S. typhi,and C. albicans*) as depicted in Fig (4). The lowest MIC value was 6.25 mg/mL for the collection strains. A summary of the MIC distribution for the three fractions against the test strains is given in Table 4. At the level of antibacterial activity, ethyl acetate fraction has the most effective value (MIC 6.25 mg/mL). It was evident from the MIC results that the lipophilicity and the low solubility profile have affected the activity of the prepared compounds and consequently their antimicrobial effect.

**Table 3.** DPPH radical scavenging activity expressed as $IC_{50}$ values (ig/mL) of various extracts from *P. tomentose*

| Extract | $IC_{50}$ |
|---|---|
| Ethanol | 0.63 (0.993)* |
| Methanol | 0.58 (0.98) |
| Ethylacetate | 0.54 (0.981) |
| Butylhydroxytoluene (BHT) | 0.61 (0.99) |

* $R^2$: Correlation coefficient

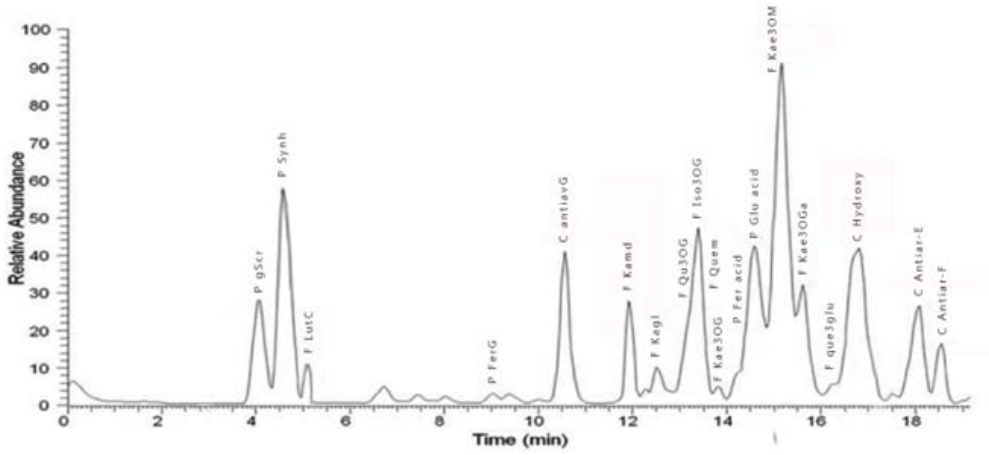


**Fig. 2.** Profile of LC-MS of *P. tomentosa* ethanolic extract. The names are according to the identified compounds in Table 2

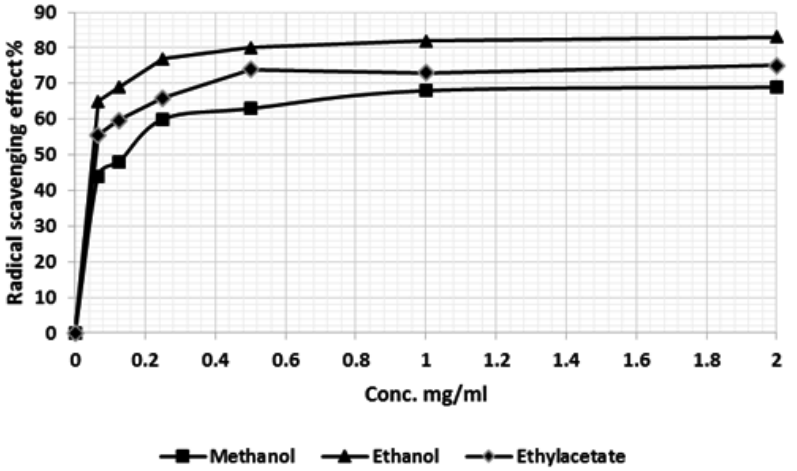


**Fig. 3.** Radical scavenging effect (%) on DPPH radicals of P. tomentosa organs

## DISCUSSION

This study has investigated the phytochemical composition and the biological properties of *Pergularia tomentosa*grown in the district of Hail. The results of metabolite profiling showed that there are 18 compounds (phenolics and cardenolides) in the extract. Fourteencompounds belonging to the phenolics and flavonoids, and 4 cardenolides were identifiedfrom leaves' aqueous extract of *P. tomentosa*. Given that *P. tomentosa* is in Asclepiadaceae, we expected that it would

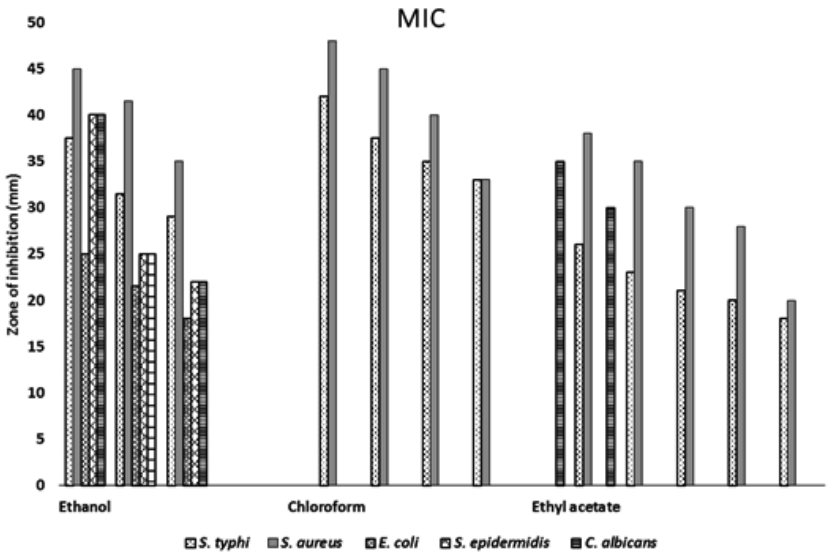


**Fig. 4.** MIC of *P. tomentosa* extracts against the pathogenic microorganisms

**Table 4.** MIC of *Pergularia tomentosa* extracts against pathogenic microorganisms

| | Conc. mg/ml | *S. typhi* | *S. aureus* | *E. coli* | *S. epidermidis* | *C. albicans* |
|---|---|---|---|---|---|---|
| Ethanol | 100 | 37.5 | 45 | 25 | 40 | 28 |
| | 50 | 31.5 | 41.5 | 21.5 | 25 | 26 |
| | 25 | 29 | 35 | 18 | 22 | 19 |
| | 12.5 | - | - | - | - | - |
| | 6.25 | - | - | - | - | - |
| Chloroform | 100 | 42 | 48 | - | - | - |
| | 50 | 37.5 | 45 | - | - | - |
| | 25 | 35 | 40 | - | - | - |
| | 12.5 | 33 | 33 | - | - | - |
| | 6.25 | - | - | - | - | |
| Ethyl acetate | 100 | 26 | 38 | - | - | 35 |
| | 50 | 23 | 35 | - | - | 30 |
| | 25 | 21 | 30 | - | - | - |
| | 12.5 | 20 | 28 | - | - | - |
| | 6.25 | 18 | 20 | - | - | - |
| Ciprofloxacin | 8 µg/ml | 25 | 28 | 24 | 30 | - |
| | 4 µg/ml | 20 | 23 | 19 | 20 | - |
| | 2 µg/ml | 15 | 14 | 13 | 18 | - |
| Fluconazole | 10 µg/ml | - | - | - | - | 17 |
| | 5 µg/ml | - | - | - | - | 13 |

have contained many cardenolides, but 79% of the identified compounds are phenolic,and 21% belong to the cardenolides. Other reports which investigated the aerial parts of this plant [9]demonstrated that its aerial parts are a rich source of flavonoids. Almost all studies showed that the roots of *P. tomentosa* are cardenolide-bearing part[6, 9]. We identified 4 cardenolides in leaves of this plant. These results suggestedthatthe leaves of *P. tomentosa* are a rich source of flavonoids and could be a suitable source for valuable cardenolides. For example, one of the identified cardenolides isGhalakinoside, which has a potent effect on cancer cells[27]. The other three identified cardenolides (Antiaroside E-G), from the resultsof Shiet al (2010), exhibited strongcardiotonic activity, with a potent inhibitor of Na+/K+-ATPase. In addition, its phenolic-compounds can play a strong antioxidant role[8, 28]. In this field, Yakubu et al (2015) and Al Jabri (2013) confirmed the antioxidant effect of the extract of this plant[2, 10, 11, 18].

The antimicrobial activity for ethanol extract was the broadest whereas the lowest MIC was 6.25 mg/mL for ethyl acetate but surprisingly the inhibition was noticed in all strains the gram-positive, gram-negative and fungus. This points to inhibitory effect applied on a metabolic pathway or a process in gene transcription especially that the collection of pathogenic microorganism has different constituents in the cell membrane and ribosomal subunits[8, 23, 29-31].

Additionally, phytochemical analysis has revealed variation in components and concentrations of *P. tomentosa* constituents grown at different agricultural regions [32].Interestingly, we can enumerate two important sources of antioxidants in *P. tomentosa*, firstly,the high concentrations of phenolic compounds in *P. tomentosa*which is considered to be a good source ofpowerful antioxidants,secondly, thehydroxyl groups in flavonoids which have the capability to react with DPPH radical by hydrogen atom donation to free radicals[7, 33], while a highly positive correlation between total phenoliccontent and antioxidant activity was established in caseof many plant species [7, 23].

## CONCLUSIONS

In conclusion, we summarize a variation between the phytochemical constituents of *P. tomentosa* plants grown in the district of Hail and other geographical regions. In addition, there are several natural phytocompounds with an antimicrobial activity could be a good target for the antimicrobial and antioxidantsindustry.

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