

## Original Research Article

# Phytochemical, antioxidant, and antimicrobial effects of methanolic leaf extract of *Rhazya stricta* decne

Ahmed Saif\*

Department of Clinical Laboratory Sciences, Faculty of Applied Medical Sciences, Najran University, Najran, Saudi Arabia

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**\*Correspondence:**

Dr. Ahmed Saif,

E-mail: [amsaif8080@gmail.com](mailto:amsaif8080@gmail.com)

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### ABSTRACT

**Background:** In this research, we investigated the antioxidant phytochemicals, antimicrobial and antibiofilm properties of methanolic leaf extract from *Rhazya stricta*.

**Methods:** The main components of *R. stricta* were confirmed through HPLC techniques. The antioxidant activity of methanolic leaf extract was examined by DPPD, ABTS, SOD and Glutathione peroxidase assays.

**Results:** The results showed that the methanolic extract of *R. stricta* showed better free radical scavenging properties with the IC50 value 74.2 mg/ml by DPPH assay. And the tested extract showed high SOD and peroxidase activities. The HPLC analysis of leaf extract reveal the presence of rutin. Antibacterial results revealed that methanolic leaf extract of *R. stricta* had higher anti-bacterial activity against multi drug resistant microbes namely, *Bacillus subtilis* (3.51 mm), *Staphylococcus aureus* (1.29 mm), *Escherichia coli* (3.39 mm), *Klebsiella pneumoniae* (2.03 mm), *Pseudomonas aeruginosa* (5.73 mm). In addition, the leaf extract showed strong antibiofilm potential against *Pseudomonas aeruginosa* at 100 µg/ml.

**Conclusions:** Overall, the methanolic leaf extract of *R. stricta* showed significant antioxidant capacity and highly inhibit the bacterial pathogens.

**Keywords:** *Rhazya stricta*, Antibacterial, Antioxidant, HPLC, Anti-biofilm activity

### INTRODUCTION

Biofilms are surface-attached ubiquitous bacterial communities encased in a matrix of extracellular polymeric substances (EPS), which consists of proteins, polysaccharides, and extracellular DNA.<sup>1</sup> Biofilms are also important for environmental survival, transmission and increased infectivity of human pathogens.<sup>2</sup> The bacteria within the biofilm are hundred times resistant than available antibiotics compared with planktonic cells.<sup>3</sup> The rapid emergence of multi drug resistant and mutagenic microbes against the existing antibiotics has necessitated the development of new antimicrobial drugs to offer effective treatment. Previously, many

antimicrobial products were identified from synthetic and natural products for the management of infectious disease-causing agents.

The medicinal value of natural products has several phytochemicals that produce a distinct physiological action on the human body.<sup>4</sup> Various botanicals have been reported to exhibit potential antioxidant activity. Antioxidants, including phenolic compounds (e.g., flavonoids, phenolic acids, and tannins), have diverse biological effects.<sup>5</sup> The Apocynaceae is a family of flowering plants and includes trees and shrubs. Apocynaceae consists of at least 5350 recognized species in 348 genera that are native to tropical and subtropical

nations.<sup>6</sup> *Rhazya stricta* grows in depressions with silty and sandy soils sometimes forming in a pure stand.<sup>7</sup> *R. stricta* is also called as “Harmal”. *R. stricta* Decne is an evergreen shrub and widely distributed in Saudi Arabia and South Asia.<sup>8,9</sup> It is a significant therapeutic plant used in indigenous medicinal herbal drugs to cure various ailments in different countries including Saudi Arabia.<sup>10</sup> The crude ethanolic extract of *R. stricta* fruit has shown anti-bacterial and lipoxygenase activities, as well as acetylcholinesterase inhibitory effect.<sup>11</sup> Also, Abdul Hameed et al highlighted that indole alkaloids from *R. stricta* exhibited anti-proliferative activity.<sup>12</sup>

The *R. stricta* extract contains numerous alkaloids, glycosides, flavonoids, tannins, and triterpenes.<sup>13</sup> Up to date around 100 indole-type alkaloids were reported from *R. stricta*. Very recently, Indole-type alkaloids isolated from *R. stricta* leaves, grown in Saudi Arabia and to evaluate their cytotoxic effect against colon cancer (HCT-116), prostate cancer (PC-3), human hepatocellular liver carcinoma (HepG2) and a normal adult African green monkey kidney cell (VERO).<sup>14</sup> More studies on pharmacological, phytochemical, toxicological and biological activities of *R. stricta* has been accounted.<sup>8,13,15-17</sup> *R. stricta* extract showed the best antioxidant, antiproliferative and antimetastatic activities at low concentration.<sup>18</sup> *R. stricta* fruits extract showed good cytotoxic effect against breast cancer cells evaluated.<sup>19</sup> This research an attempt has been made to develop antimicrobial drug from native plant *R. stricta* against different pathogens and the presence of rutin in the plant extract was confirmed by HPLC. Finally, we evaluated the antioxidant and antibiofilm properties of *R. stricta* against *Pseudomonas aeruginosa*.

## METHODS

### Plant material and extraction

The leaves of *Rhazya stricta* taken from Southwest part of Saudi Arabia (Abha) during the month of October 2019. The leaves were then dried at room temperature after the process of washing with distilled deionized water (DDH<sub>2</sub>O). Aqueous solution of *R. stricta* was obtained by dissolving finely powdered *R. stricta* leaves (10 g) in 100 ml of DDH<sub>2</sub>O.<sup>20</sup> The extracted solution were then send the main research laboratory CLS in Najran University (NU), to start studying the antioxidant, antibacterial and anti-biofilm activity. Whereas, the quantification of rutin using RP-HPLC was done in KSU business centre lab College of pharmacy then analyse the data in NU main research lab.

### Antioxidant activity of *Rhazya stricta*

DPPH free radical scavenging activity: following the method by Shaltout et al the antioxidant activity *R. stricta* extract was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH.<sup>7</sup> About 0.4 ml of 0.1 mM DPPH solution was added to

200 µl of *R. Stricta* extract. Then, the sample was kept for 30 min at room temperature. The absorbance of the sample was measured at 517 nm against blank (methanol). Ascorbic acid (vitamin C) was used as a positive control. The radical scavenging activity was calculated using the formula:

$$\text{DPPH scavenged (\%)} = (\text{blank} - \text{sample}) / (\text{blank}) \times 100$$

ABTS free radical scavenging activity: the radical cation derived from ABTS was prepared by the reaction of 60 mM ABTS solution with 0.3 M Manganese dioxide solution in 0.1 M phosphate buffer, pH 7. Then, the mixture was shaken, centrifuged, filtered, and the absorbance (Ablank) of the resulting green-blue solution (ABTS radical solution) was measured at wavelength 734 nm.<sup>21</sup> Then, 50 µl of test plant extract in phosphate buffered methanol was added. The absorbance (Asample) was measured. The reduction in color intensity was expressed as % inhibition. The % inhibition for each compound is calculated from the following equation:

$$\text{Inhibition\%} = (\text{blank} - \text{sample}) / (\text{control}) \times 100$$

Ascorbic acid was used as standard antioxidant (positive control). Blank sample was run without ABTS and using MeOH/phosphate buffer (1:1) instead of sample. Negative control sample was run with MeOH/phosphate buffer (1:1) instead of tested compound. The data are expressed as mean±SEM for three independent experiments.

Superoxide dismutase (SOD)-like radical scavenger activity: following the method by Obaid et al this assay relies on the ability of the extract to inhibit the PMS-mediated reduction of NBT dye.<sup>8</sup> The SOD reaction mixture had a final volume of 3.0 ml. It was composed of 0.5 ml of 0.3 mmol NBT, 0.5 ml of 0.47 mmol NADH, 1.8 ml of 0.1 M sodium pyrophosphate buffer, 0.1 ml of tested plant extract and 0.1 ml of 0.093 mmol PMS. The reaction was initiated by the addition of PMS and the increase in absorbance at 560 nm was followed with a recording spectrophotometer for 5 min. The percent inhibition of free radical formation (I) was calculated as

$$\text{Inhibition\%} = (\text{blank} - \text{sample}) / (\text{control}) \times 100$$

where A control is the change in absorbance at 560 nm over 5 min following the addition of PMS to the reaction mixture in the absence of ligand or complex and Atest is the change in absorbance at 560 nm over 5 min following the addition of PMS to the reaction mixture in the presence of ligand or complex.

Glutathione peroxidase like activity: GPx kit (Biodiagnostic, Egypt) was used for the determination of GPx according to Paglia et al.<sup>22</sup> The reaction mixture contained 1 ml assay buffer (50 mM phosphate buffer containing 0.1% triton X-100) and 0.1 ml NADPH reagent (24 µmol Glutathione, 12-unit glutathione

reductase and 4.8  $\mu\text{mol}$  NADPH) and 0.01 ml (41  $\mu\text{M}$ ) tested extract and the reaction was started by the addition of  $\text{H}_2\text{O}_2$  (0.8 mM). The contents were mixed well, and the absorbance was recorded at 340 nm over a period of 3 min against deionized water. The change of absorbance per minute (A340nm / min) was estimated using ebselen (41  $\mu\text{M}$ ) as the positive control. In case of colored extracts, their activities were estimated after subtracting their own absorbance at the used wavelength.

#### Quantification of quercetin and rutin by reverse phase-HPLC (RP-HPLC)

The rutin content was examined through RP-HPLC according to the method of Balasubramanian et al with minor modifications. The quantification of rutin was examined by a Waters 2998 liquid chromatography (Waters, Milford, MA) equipped with the photodiode array (PDA) detector at the wavelength of 375 nm, and all the data were processed with Empower 2 software. 20  $\mu\text{L}$  of filtered sample and standard were injected into the Symmetry® C18 column (4.6 mm $\times$ 250 mm, 5  $\mu\text{m}$ ) and eluted isocratically with HPLC-grade methanol: water (with 0.1% orthophosphoric acid) in a ratio of 80:20 v/v at a flow rate of 1.0 ml/min. The quantity of rutin present in each sample was estimated by comparing the standard peak area with sample area peak. The solutions (standard and sample) were injected in triplicate and the quercetin content was quantified following the method described by Balasubramanian and his colleagues.<sup>23</sup>

#### Antibacterial activity

Antibacterial activity of *R. stricta* extracts was carried out by agar well diffusion method.<sup>24</sup> Antimicrobial activity of the different concentrations of *R. stricta* extracts was tested against the selected pathogens *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*. Amikacin (chloramphenicol) was used as positive control. As per the standard protocol, the wells size (7 mm) was made on the Muller-Hinton agar plates with bacterial lawn. Petri plates were kept for incubation at room temperature for 24 h. After 24 h of incubation, the zones of inhibition were measured, and three replicates were maintained.

#### Anti biofilm activity

Anti biofilm assay was executed by using different concentrations of *R. stricta* extracts (25, 50, 75, 100  $\mu\text{g/ml}$ ). The biofilm shaping property was confirmed by polystyrene tissue culture plate (PTCP) technique. The pathogen *P. aeruginosa*, was inoculated into 10 ml of trypticase soy soup with 2% (w/v) glucose and seeded on 1 $\times$ 1 cm<sup>2</sup> cover slide. After inoculation, the PTCP was incubated for 24 h and the cover slide was washed with sterilized double distilled water and was stained with 0.2% (v/v) crystal violet stain. After the staining, the biofilm formation was observed under the light microscope (Nikon Ni-E, NIS Elements BR software,

DS-2/3 Digital Camera) at 40X magnification. By using micro-ELISA auto Epoch microplate spectrophotometer (model BioTek) the quantification and percentage of biofilm inhibition (stained adherent) was measured at 570 nm wavelength.<sup>24</sup>

#### Quantification of extra polysaccharide (EPS) production

*P. aeruginosa* was cultured in LB broth with the presence and/or absence of *R. stricta* extracts at different concentrations (25-100  $\mu\text{l}$ ) for quantification and extraction of EPS. Biofilms adhered to the walls of test tubes were gathered during late log-phase by vigorous shaking, centrifuged at 10,000 rpm for 30 min at 4 °C and the supernatant was filtered using 0.22  $\mu\text{m}$  nitrocellulose membrane. Followed by the method of Stepanović et al the filtered supernatant was then suspended with the thrice volume of 100% ice-cold ethanol and incubated overnight at 4°C to precipitate the EPS.<sup>25</sup> The EPS production was quantified following the method described by Huston et al.<sup>26</sup>

#### Data analysis

Statistical analyses were done using GraphPad Prism version 5.04 and version 6 software (GraphPad Software, San Diego, California, USA, www.graphpad.com). One-way or two-way ANOVA was used as appropriate.

## RESULTS

#### Antioxidant activity

ABTS<sup>•+</sup> scavenging activity; in the ABTS scavenging assay, the ethanolic extract of *R. stricta* highly scavenged ABTS<sup>•+</sup> with IC<sub>50</sub> value was 45.9 $\pm$ 3.8%, which was lower than Ascorbic acid (76.4 $\pm$ 5.9%) (Table 1). This result revealed that the Harmal extract has moderate ABTS<sup>•+</sup> scavenging activity.

**Table 1: ABTS free radical scavenging activity.**

Treatment	IC <sub>50</sub> ( $\mu\text{g/ml}$ )
<i>Rhazya stricta</i> extract	69.3 $\pm$ 7.1
Ascorbic acid	88.6 $\pm$ 7.2

**Table 2: DPPH free radical scavenging activity.**

Treatment	IC <sub>50</sub> ( $\mu\text{g/ml}$ )
<i>Rhazya stricta</i> extract	74.2 $\pm$ 7.1
Ascorbic acid	89.1 $\pm$ 7.2

**Table 3: SOD like radical scavenger activity.**

Treatment	IC <sub>50</sub> ( $\mu\text{g/ml}$ )
<i>Rhazya stricta</i> extract	55.5 $\pm$ 3.6
Ascorbic acid	77.3 $\pm$ 6.3

**Table 4: Glutathione peroxidase like activity.**

Treatment	0 min	3 min	ΔA/T	ΔA/T* 10 <sup>-3</sup>
<b>Rhazya stricta extract</b>	0.132	0.121	0.003667	3.7±0.31

DPPH• scavenging activity; the DPPH radical scavenging properties of ethanol extract of *R. stricta* and Ascorbic

acid was determined. The DPPH radical scavenging effect was compared with synthetic antioxidant, Ascorbic acid. The extract of *R. stricta* exhibited better free radical scavenging properties with the IC50 value 74.2 mg/ml when compared to standard Ascorbic acid (89.1 mg/ml) (Table 2). However, the radical scavenging effect of *R. stricta* was negligible than that of standard (vitamin C). This result showed that the *R. stricta* extract was able to inhibit oxidative stress caused by DPPH.

**Table 5: Antibacterial activity of *R. stricta* extract.**

Pathogens	Pathogens treated with <i>Rhazya stricta</i> extract zone of inhibition (mm)				
	Concentration				
	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml	Positive control (C 30 µg)
<i>B. subtilis</i>	0.21±0.03	1.54±0.01	2.03±0.06	3.51±0.09	9.22±0.46
<i>S. aureus</i>	0.12±0.03	1.85±0.31	2.03±0.23	1.29±0.21	10.09±0.84
<i>E. coli</i>	0.17±0.02	0.47±0.03	1.23±0.18	3.39±0.15	9.27±0.87
<i>K. pneumoniae</i>	0.19±0.03	1.03±0.06	1.04±0.14	2.03±0.12	7.02±0.73
<i>P. aeruginosa</i>	0.88±0.13	1.67±0.17	3.43±0.47	5.73±0.49	11.08±0.13

Positive control: C-chloramphenicol (30 µg); values are mean ±standard deviation of five replicates, within a row, different ANOVA letters indicate significant differences (p<0.05).

**Table 6: Antibiofilm activity of *R. stricta* extract.**

Concentration (µg/ml)	Biofilm activity (% of inhibition)	Exopolysaccharide production (EPS % of inhibition)
<b>Control</b>	0.0±0.00	0.0±0.00
<b>25</b>	19.03±1.14	20.02±1.03
<b>50</b>	41.33±2.04	39.05±1.12
<b>75</b>	63.41±2.03	58.12±2.21
<b>100</b>	86.12±3.03	85.17±3.30

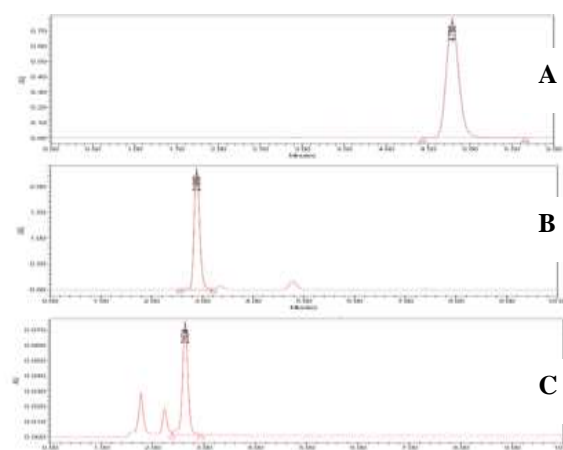
Values are mean±standard deviation of five replicates. Within a row, different ANOVA letters indicate significant differences (p<0.05).

SOD-like activity assay; in this research, PMS assay was adopted to assess the SOD scavenging activity of *R. stricta* was depicted in Table 3. This result shows that the percentage of SOD activity of methanol extract of *R. stricta* was 55.5±3.6, while Ascorbic acid achieved 76.4±5.9%, respectively. However, this value was lower than that of Ascorbic acid. Glutathione peroxidase-like activity assay; the NADPH-reductase coupled assay determined that the *R. stricta* extract had a potent glutathione peroxidase activity of 3.7±0.31 µg/ml (Table 4).

**HPLC analysis**

The flavonoids of the *R. stricta* extract were studied qualitatively using High Performance Liquid Chromatography. The conditions for HPLC such as mobile phase composition, temperature and flow rate were optimized to accomplish a good resolution and symmetrically shaped peak for rutin in less run-time. The results of HPLC analysis confirmed that the quercetin and rutin were using reference standard. The rutin compound

was present in *R. stricta* plant extracts (3.03 mg/g) (Figure 1).

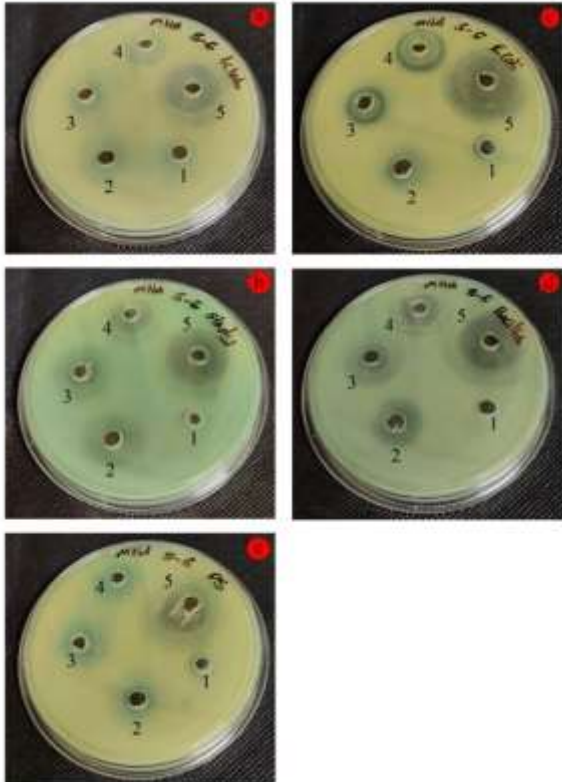


**Figure 1: HPLC chromatograms of quercetin and rutin in the *R. Stricta* extract, A) Standard of quercetin, B) Standard of rutin, C) methanolic extract of from *R. Stricta* extract.**



**Antibacterial activity**

The antibacterial effects of methanolic extracts of *R. stricta* on the five selected bacterial pathogens namely, *B. subtilis*, *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa* was evaluated in the present research. The methanolic extracts of *R. stricta* were highly inhibiting the growth of *B. subtilis* (3.51 mm) followed by *S. aureus* (1.29 mm), *E. coli* (3.39 mm), *K. pneumoniae* (2.03 mm), and *P. aeruginosa* (5.73 mm) at 100 µg/ml respectively (Table 5, Figure 2).

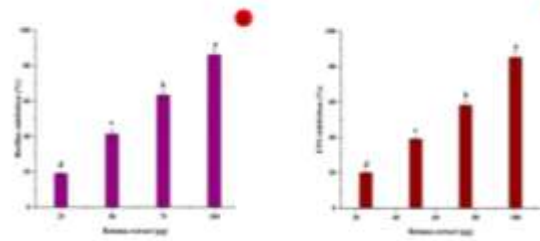


**Figure 2: Antimicrobial activity zone on plate formethanolic extract of *Rhazya stricta* extract.**

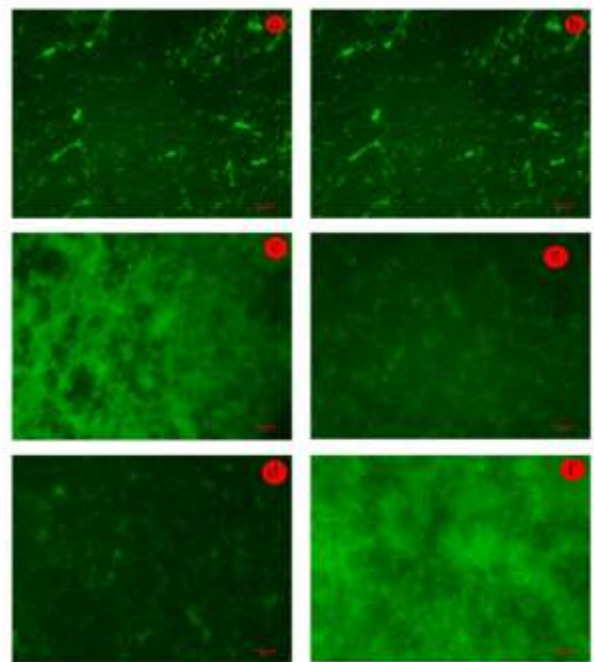
**Anti-biofilm activity**

In this research, we investigated the anti-biofilm activity of methanolic *R. stricta* extracts against *Pseudomonas aeruginosa* by biofilm inhibition assay. Our results, showed a maximum inhibitory effect of *R. stricta* extracts, were almost 85% for *P. aeruginosa* at 100µg/mL (Table 6, Figure 3). Light microscopic results showed high number of cell death and cell wall dispersion after treatment with 100µg/ml of *R. stricta* extract (Figure 4). Notably, very limited numbers of scattered cell aggregates were observed in the biofilms and there were less viable cells in the aggregates after 24h exposure to the *R. stricta* extracts. Our results indicated that increasing concentration of plant extract, lead to the dispersion of biofilms and finally were left with single planktonic cells on the slide. Also, the plant

extract not only led to the disintegration of biofilm, but also reduced the surface area and thickness of biofilm.



**Figure 3 Anti-biofilm activity of methanolic extract of *Rhazya stricta* extract against *Pseudomonas aeruginosa*, a) Biofilm quantification Spectrophotometer assay, b) methanolic extract of *Rhazya Stricta* affects extracellular polymeric substances (EPS) production in *Pseudomonas aeruginosa*. Values are mean±standard deviation of five replicates. Within a column, different ANOVA letters indicate significant differences (p<0.05).**



**Figure 4: Fluorescence Microscopy observation (×40) of *Pseudomonas aeruginosa* adhesion phases on the glass surfaces with different concentration (1 mg/ml) of methanolic extract of *Rhazya stricta* plant. a, b) control biofilm slide without extract, c) extract treated at 25 µl, d) extract treated at 50 µl, e) extract treated at 75µl, f) extract treated at 100µl.**

**Quantification of *exo poly substances (EPS) inhibition***

Herein, we studied the impact of *R. stricta* extracts on EPS formed by of *P. aeruginosa* within biofilm. From the results, we observed that the *R. stricta* extracts

significantly reduced the formation of EPS in *P. aeruginosa* strains at all different concentration of plant extracts used. The reduction in exopolysaccharide (EPS) synthesis was found not only to inhibit the biofilm formation but also to remove the preformed biofilms effectively, although this effect was found to be dose dependent on the specificity of the EPS matrix of biofilms (Table 6). Our results clearly indicated that the plant extract effectively reduce the EPS production at low concentration when compared with other subsequent concentrations.

## DISCUSSION

In this research, the focus was on comprehensive antioxidant phytochemical profiling of *R. stricta* by HPLC. Analytical HPLC technique was conducted to separate and detect individual polyphenol in methanol leaf extract of *R. stricta*. The biologically active entity is often present only as minor component in the extract and the resolving power of HPLC is ideally suited to the rapid processing of such multicomponent samples on both, an analytical and preparative scale. The chromatogram showed the presence of rutin was identified. The peak rutin in the sample chromatograms was confirmed by spiking with its standard and determination of retention time (Figure 4). Similarly, Aziz et al noticed the presence of alkaloids, namely rhazine, vincamine, yohimbine from *R. stricta* through HPLC-MS, while Akhgari et al reported that the indole alkaloids from *R. stricta* by ultra-performance liquid chromatography-mass spectrometry UPLC-MS.<sup>13,27</sup>

In this present study we confirmed the presence of flavonoid rutin in the *R. stricta* extract by HPLC. Flavonoids are well known scavenging agents against free radicals associated with oxidative damage.<sup>28</sup> Free radicals can easily cross cell-membranes, readily react with most biomolecules in cells, as well as inflict tissue damage or cause cell death.<sup>29</sup> In this research, the flavonoid rich *R. stricta* extract exhibited potent antioxidant activities by DPPD, ABTS, SOD and Glutathione peroxidase assays. Methanolic extract would have the maximum activity, showing that its metabolites in the plant with the most radical-scavenging activity are of medium polarity. For instance, Razali et al noticed that methanolic shoot extract of *Anacardium occidentale* showed higher antioxidant activity compared with ethyl acetate and hexane extracts as determined through ABTS, DPPH, superoxide anion, and NO radical scavenging assays.<sup>30</sup>

DPPH is a stable free radical has been widely used in phytomedicine for the assessment of scavenging properties. The *R. stricta* leaf methanolic extract demonstrated H-donor activity. The methanol extract of *R. stricta* showed good anti-DPPH activity (IC<sub>50</sub> = 74.2 mg/ml) compared to reference compound. The result agreed with earlier studies, Al-Busafi et al. (2007) who has highlighted that the DPPH radical scavenging activity

of n-BuOH extracts of nine medicinal plants, among them *Aristolochia bracteolata* (-57.8%), *Citrullus colocynthis* (-39.6%), and *R. stricta* (-31.3%) and *Nerium oleander* (-37.2%) had moderate radical scavenging property.<sup>31</sup> ABTS is an excellent substrate for peroxidases and is frequently used to study the antioxidant properties of natural compounds.<sup>32</sup> The methanol leaf extract of *R. stricta* showed maximum ABTS (69%) scavenging property compared to Ascorbic acid (89%) respectively. A similar outcome was reported by Shahinuzzaman et al that *Ficus carica* showed the highest antioxidant activity of 83.04%±2.16% in ABTS. The ethyl acetate fraction of *Clerodendrum cyrtophyllum* Turcz exhibited potent ABTS radical scavenging activity with IC<sub>50</sub> value of 23.00±1.5 µg/ml respectively.<sup>33</sup>

The results of SOD and Glutathione peroxidase displayed that the *R. stricta* extract had potential SOD and peroxidase activities, as these enzymes play a vital role in antioxidant defense. Comparably, Kumar et al who have reported that the antioxidant enzymes superoxide dismutase, catalase, ascorbate peroxidase, glutathione reductase and polyphenol oxidase were significantly higher in *Cassia fistula* followed by *Cinnamomum cassia*, *Acacia catechu* and *Citrus limon* respectively.<sup>34</sup> The tolerant genotype VA13 of *Amaranthus tricolor* showed higher catalase (CAT), superoxide dismutase (SOD) enzyme activity.<sup>35</sup>

Our results clearly reveal that *R. stricta* ethanolic extract disturb pathogen growth by cell wall disruption. Herein, the methanolic extract of *R. stricta* had relevant bacteria growth inhibition on the five selected species of public health importance. At 100 µg/ml, *R. stricta* had inhibitory potential against *B. subtilis* (3.51 mm) followed by *S. aureus* (1.29 mm), *E. coli* (3.39 mm), *K. pneumoniae* (2.03 mm), and *P. aeruginosa* (5.73 mm) (Table 5). Similarly, Shegute and Washhun (2020) studied the leaf extracts of *Agava americana* highly effective on *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella species*, and *Eshercia coli*.<sup>36</sup> The ethanolic root extract of *Plumbago zeylanica* showed good antimicrobial activities on *V. cholerae*, *E. coli*, *P. aeruginosa*, *Curvularia lunata*, *Colletotrichum corchori*, and *Fusarium equiseti*.<sup>37</sup>

In addition, the antibiofilm activity of *R. stricta* leaf extract on *P. aeruginosa* by biofilm inhibition assay. Anti-biofilm results showed a maximum inhibitory effect of *R. stricta* leaf extract, were almost 86% for *P. aeruginosa* at 100 µg/ml. The fluorescence Microscopy results, revealed a wide spectrum of morphological differences in biofilm topography and architectures. El-Bashiti et al studied the antibiofilm property of five-plant extract against *E. coli*, *S. aureus*, *P. aeruginosa* and *K. pneumoniae*.<sup>38</sup> The aqueous extracts of the *Punica granatum*, *Ricinus communis*, and *Allium sativum* had strong anti-biofilm activity on *Streptococcus mutans*,<sup>39</sup> while Gomes and his colleagues observed that hydro

methanolic extract of *Eucalyptus globulus* was highly effective against *Staphylococcus aureus*.<sup>40</sup>

Although the results in this study seemed to be promising with regards antioxidant and antimicrobial property of the *R. stricta*, the toxicity, molecular effect and other proteomic studies need to be done to clarify the mode of action of the leaf extract and the pharmacological safety of the usage.

## CONCLUSION

The emerging multi-drug resistance microbes provide a major obstacle for the management of microorganisms. Therefore, the development effective drug is crucial for public health. In this research, we found out that the leaf extract of *R. stricta* showed good antioxidant property. Moreover, this plant extracts had high antimicrobial and anti-biofilm active against the selected pathogens. Overall, we conclude that the methanolic extract of *R. stricta* could be used as alternative agent on clinical pathogens.

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*Ethical approval: The study was approved by the Institutional Ethics Committee*

## REFERENCES

- Franklin MJ, Nivens DE, Weadge JT, Howell PL. Biosynthesis of the *Pseudomonas aeruginosa* Extracellular Polysaccharides, Alginate, Pel, and Psl. *Front Microbio.* 2011;2:14-9.
- Parsek MR, Singh PK. Bacterial Biofilms: An Emerging Link to Disease Pathogenesis. *Annu Rev Microbiol.* 2003;57(1):677-701.
- Mah TFC, O'Toole GA. Mechanisms of biofilm resistance to antimicrobial agents. *Trends in Microbiology.* 2001;9(1):34-9.
- Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. *Afr J Biotechnol.* 2005;4(7):685-8.
- Chung KT, Wong TY, Wei CI, Huang YW, Lin Y. Tannins and Human Health: A Review. *Crit Rev Food Sci Nutr.* 1998;38(6):421-64.
- Kadereit JW, Bittrich V, eds. Flowering Plants. Eudicots: Apiales, Gentianales (except Rubiaceae). United States of America: Springer International Publishing; 2018.
- Shaltout KH, El-Sheikh MA. Vegetation of the urban habitats in the Nile Delta region, Egypt. *Urban Ecosystems.* 2002;6(3):205-21.
- Obaid AY, Voleti S, Bora RS. Cheminformatics studies to analyze the therapeutic potential of phytochemicals from *Rhazya stricta*. *Chem Central J.* 2017;11(1):11.
- Bukhari NA, Al-Otaibi RA, Ibrahim MM. Phytochemical and taxonomic evaluation of *Rhazya stricta* in Saudi Arabia. *Saudi J Biol Sci.* 2017; 24(7):1513-21.
- Gilani SA, Kikuchi A, Shinwari ZK, Khattak ZI, Watanabe KN. Phytochemical, pharmacological and ethnobotanical studies of *Rhazya stricta* Decne. *Phytother Res.* 2007;21(4):301-7.
- Sultana N, Khalid A. Phytochemical and enzyme inhibitory studies on indigenous medicinal plant *Rhazya stricta*. *Natural Product Res.* 2010;24(4):305-14.
- Abdul-Hameed ZH, Alarif WM, Omer AMS, et al. Selective Anti-proliferative Activity of Indole Alkaloids from *Rhazya stricta* Decne Leaves. *LOC.* 2019;16(12):941-7.
- Aziz AT, Alshehri MA, Alanazi NA. Phytochemical analysis of *Rhazya stricta* extract and its use in fabrication of silver nanoparticles effective against mosquito vectors and microbial pathogens. *Sci Total Environ.* 2020;700:134443.
- Abdul-Hameed ZH, Alarif WM, Sobhi TR. New cytotoxic indole-type alkaloids obtained from *Rhazya stricta* leaves. *South African J Botany.* 2021;137:298-302.
- Mahmood R, Kayani WK, Ahmed T. Assessment of antidiabetic potential and phytochemical profiling of *Rhazya stricta* root extracts. *BMC Complement Med Ther.* 2020;20(1):293.
- Asiry KA, Zaitoun AA. Evaluation of the toxicity of three plant extracts against the Khapra beetle *Trogoderma granarium* Everts (Coleoptera: Dermestidae) under laboratory conditions. *Revista de la Sociedad Entomológica Argentina.* 2020;79(1).
- Ali BH, Alqarawi AA, Bashir AK, Tanira MO. Antioxidant action of extract of the traditional medicinal plant *Rhazya stricta* Decne. in rats. *Phytother Res.* 2000;14(6):469-71.
- Al-Dabbagh B, Elhaty IA, Al Hroust A, et al. Antioxidant and anticancer activities of *Trigonella foenum-graecum*, *Cassia acutifolia* and *Rhazya stricta*. *BMC Complement Altern Med.* 2018;18(1): 240.
- Al-Zharani N, Abutaha A. Apoptotic Induction and Anti-Migratory Effects of *Rhazya Stricta* Fruit Extracts on a Human Breast Cancer Cell Line. *Molecules.* 2019;24(21):3968.
- Murugan K, Samidoss CM, Panneerselvam C. Seaweed-synthesized silver nanoparticles: an eco-friendly tool in the fight against *Plasmodium falciparum* and its vector *Anopheles stephensi*? *Parasitol Res.* 2015;114(11):4087-97.



21. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine.* 1999;26(9-10):1231-7.
22. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med.* 1967;70(1):158-69.
23. Balasubramanian M, Anbumegala M, Surendran R, Arun M, Shanmugam G. Elite hairy roots of *Raphanus sativus* (L.) as a source of antioxidants and flavonoids. *3 Biotech.* 2018;8(2):128.
24. Vanaraj S, Keerthana BB, Preethi K. Biosynthesis, Characterization of Silver Nanoparticles Using Quercetin from *Clitoria ternatea* L to Enhance Toxicity Against Bacterial Biofilm. *J Inorg Organomet Polym.* 2017;27(5):1412-22.
25. Stepanović S, Vuković D, Hola V. Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. *Apmis.* 2007;115(8):891-9.
26. Huston AL, Methe B, Deming JW. Purification, Characterization, and Sequencing of an Extracellular Cold-Active Aminopeptidase Produced by Marine Psychrophile *Colwellia psychrerythraea* Strain 34H. *Appl Environ Microbiol.* 2004;70(6):3321-8.
27. Akhgari A, Laakso I, Seppänen-Laakso T. Analysis of Indole alkaloids from *Rhazya stricta* hairy roots by ultra-performance liquid chromatography-mass spectrometry. *Molecules.* 2015;20(12):22621-34.
28. Ferguson PJ, Kurowska EM, Freeman DJ, Chambers AF, Koropatnick J. In vivo inhibition of growth of human tumor lines by flavonoid fractions from cranberry extract. *Nutr Cancer.* 2006;56(1):86-94.
29. Yuan JF, Zhang ZQ, Fan ZC, Yang JX. Antioxidant effects and cytotoxicity of three purified polysaccharides from *Ligusticum chuanxiong* Hort. *Carbohydrate Polymers.* 2008;74(4):822-7.
30. Razali N, Razab R, Junit SM, Aziz AA. Radical scavenging and reducing properties of extracts of cashew shoots (*Anacardium occidentale*). *Food Chemistry.* 2008;111(1):38-44.
31. Al-Busafi S, Al-Riyami M, Al-Ouwaisi K, Hisham A. Screening of Antioxidant and Radical Scavenging Activities of Some Omani Medicinal Plants. *SQU J Sci.* 2007;12(1):1.
32. Reszka KJ, Britigan BE. Doxorubicin inhibits oxidation of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) by a lactoperoxidase/H<sub>2</sub>O<sub>2</sub> system by reacting with ABTS-derived radical. *Arch Biochem Biophysics.* 2007;466(2):164-71.
33. Shahinuzzaman M, Yaakob Z, Anuar FH. In vitro antioxidant activity of *Ficus carica* L. latex from 18 different cultivars. *Sci Rep.* 2020;10(1):10852.
34. Kumar U, Mishra M, Prakash V. Assessment of antioxidant enzymes and free radical scavenging activity of selected medicinal plants. *Free Radicals Antioxidants.* 2012;2(3):58-63.
35. Sarker U, Oba S. Catalase, superoxide dismutase and ascorbate-glutathione cycle enzymes confer drought tolerance of *Amaranthus tricolor*. *Sci Rep.* 2018; 8(1):16496.
36. Shegute T, Wasihun Y. Antibacterial Activity and Phytochemical Components of Leaf Extracts of *Agave americana*. *Journal of Experimental Pharmacology.* 2020;12:447-4.
37. Chuah EL, Zakaria Z, Suhaili Z, Bakar SA, Desa MNM. Antimicrobial activities of plant extracts against methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*. *J Microbiol Res.* 2014;4:6-10.
38. El-Bashiti TA, Masad AA, Mosleh FN, Madi YMA. Antibacterial and Anti-Biofilm Activity of selected plant extracts against some human pathogenic microorganisms. *Pakistan J of Nutr.* 2019;18(11):1014-20.
39. Shams S, Mehdipour A, Kermani S, Ghorbani H, Ragolia S. Anti-Biofilm Activity of *punica granatum*, *ricinus communis*, and *allium sativum* plant extracts on *Streptococcus mutans*. *IEM.* 2018;4(2):67-72.
40. Gomes F, Martins N, Barros L. Plant phenolic extracts as an effective strategy to control *Staphylococcus aureus*, the dairy industry pathogen. *Industrial Crops Products.* 2018;112:515-20.

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