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HMI and BHA conceived and designed the study. HMI, AMSA, AAH, QYA, AAT, BHA performed the experiments. HMI analyzed data, wrote and revised the paper.

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Phytochemical Screening, Antioxidant and Antimicrobial Activities of *Aloe rubroviolacea* Schweinf.

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Abstract:

The purpose of this study was to investigate the phytochemical content, antioxidant capacity, and antibacterial efficacy of *Aloe rubroviolacea* leaf extracts. Methanol and aqueous leaf extracts (leaf skin and gel) were created using the rotary shaker method. Phytochemical analysis reveals the presence of Alkaloids, Saponins, Tannins, Phenol, Steroids, Reducing sugar, Flavonoids, and Carbonly in *A. rubroviolacea* methanol leaf (skin and gel) extracts, while; Alkaloids, Saponins, Tannins, Reducing sugar, Flavonoids, and Carbonyl compounds, were recorded in aqueous leaves skin extract, whereas; Alkaloids, Saponins, Reducing sugar, Flavonoids, and Carbonyl compounds were found in aqueous gel extract. The FT-IR spectrum analysis exhibits the presence of Hydroxyl group (OH), C-H aliphatic, C=C aromatic, carbonyl group (C=O), and C-O group in the methanol leaf skin and gel extracts. Furthermore, the presence of accumulated double bonds / isocyanates and isothiocyanates was reported in the methanol gel extract. On the other hand, the FT-IR spectrum analysis displays the presence of the Hydroxyl group (OH), and methane group (C-H) in the aqueous leaf (skin and gel) extracts, while N-H, CH_3, and C-N, were only recorded in aqueous gel extract. The antioxidant activity of *A. rubroviolacea* methanol (leaf skin and gel) and aqueous (leaf skin and gel) extracts was investigated using the DPPH radical scavenging activity method. The methanol leaf skin and aqueous gel extracts exhibit a high antiradical activity towards DPPH radical when compared to the aqueous leaf skin and methanol gel extracts. The four extracts were tested for antimicrobial activity against four human pathogenic bacteria (*Staphylococcus aureus*, *Streptococcus pneumonia*, *Escherichia coli*, and *Pseudomonas aeruginosa*) and one fungal pathogen; *Candida albicans*, using the agar well diffusion technique. The methanol leaf skin extract was the most effective against all tested pathogens, followed by the methanol gel extract. The high antimicrobial activity of the methanol leaf skin extract could be suggested to the presence of high antioxidant components such as, phenolics, flavonoids & tannins compounds, and rhodoxanthin pigment in the extract.

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INTRODUCTION

Aloe is a large genus in the family Asphodelaceae, subfamily Alooideae (Takhtajan, 2009), comprising over 600 taxa; including subspecies and varieties (Carter *et al*., 2011; Grace *et al*., 2011). All *Aloe* species are perennial, leaf-succulent xerophytes that are adapted to survive in regions with low or unpredictable rainfall. Most aloes have thick and fleshy leaves that are enlarged to store water. Members of the *Aloe* genus may be acaulescent (with very short stems that are completely concealed by rosulate leaves) or caulescent. Flowers are produced on racemose inflorescences, which can be simple or branched (Newton, 2020; Reynolds, 2004; Ibrahim *et al.*, 2022).

Most *Aloe* species are located in southern Africa and eastern parts of the continent, with very few species recorded in West African countries. Many other species are found on the Arabian Peninsula (which is closely connected to species of northeast Africa), Madagascar, and some of the smaller Indian Ocean islands (Reynolds, 2004; Carter *et al.*, 2011; Ibrahim *et al.*, 2022). Based on Reynolds (2004) and Carter *et al.* (2011), many countries have some endemic *Aloe* species, Madagascar for instance (all the recorded 77 Aloe species are endemic; therefore, endemism is 100%) has the highest rate of endemism followed by South Africa (71 endemic *Aloe* species from total 119 *Aloe* species – 60% endemism). Certain species from the genus *Aloe* have been used as therapeutic plants for centuries, especially *A. vera*, which was mentioned in the herbal of Dioscorides (produced in the first century CE) and in the Codex Aniciae Julianae (produced in the year CE 512). Moreover, *Aloe* species have also been used in Islamic traditional medicine (ITM) for a very long time (Reynolds, 2004; Akaberi *et al.*, 2016).

Only a few species of *Aloe* have been used in folk remedies and pharmaceutical uses, among them *A. vera* which is considered the most widely studied and commercialized species. Based on the findings of several vitro and in vivo pharmacological studies and clinical trials, *Aloe* spp. displayed a variety of pharmacological properties confirmed with the Ethnomedical uses of Aloes such as anti-ulcer activities, and wound healing. Moreover, according to the outcomes of modern pharmacological investigations, Aloes show new medicinal properties such as anti-cancer, antimicrobial, anti-diabetic, and hypoglycaemic (Akaberi *et al*., 2016). Secondary metabolites are produced by all plants as a result of primary metabolites. Secondary metabolite chemical compounds are frequently specific and confined to taxonomically related species and they provide non-essential roles in plants. Those compounds (glycosides, alkaloids, volatile oils, flavonoids, plant phenols, tannins, and terpenoids.) are utilized for defense and protection against predators and infections (Singh, 2010; 2016). Due to the presence of polyphenols (e.g flavonoids and tannins) and phenols, aloes have cytotoxic capabilities and can damage pathogenic cells, therefore they possess antimicrobial activity (Hamman, 2008; Lawrence, *et al.*, 2009; Ibrahim *et al.*, 2022). *Aloe* species are grown in Yemen among them 13 species endemic to yemen, and 7 species are endemic to Arabian Peninsula including *Aloe rubroviolacea* Schweinf (Al Khulaidi, 2013). *Aloe rubroviolacea* Schweinf is an armed, trailing stem *Aloe*, commonly pendulous on cliffs, leaves, grey reddish-violet arranged in a dense cluster at the apex of the stem, flowers red carried on erect branched inflorescence / or grown on the pendulous recurved inflorescence (Wood, 1997; Carter *et al.*, 2011). *A. rubroviolacea* occurs on high mountain rocks and cliffs, above 2500m, along the whole length of Yemen escarpment north into Saudi Arabia near Abha but is absent from mountains with an annual rainfall of less than 500mm. (Wood, 1983; Carter *et al.*, 2011).

Earlier studies have focused mainly on the antimicrobial effect of *A. vera*, while research on the antimicrobial influence of *A. rubroviolacea* has been comparatively less detectable; thus, this work aims to investigate the phytochemical, antioxidant, and antimicrobial activities of methanol and aqueous extracts of *A. rubroviolacea* leaf (skin and gel) grown in Yemen.

MATERIAL AND METHODS

Collection and Preparation of plant extract

Plant samples (Figure 1) were gathered from Alahjer (latitude: 15°27'39 N, longitude: 43°52' 12 E, and altitude: 2321m asl.) region, Al-Mahweet government, between September 21, 2022, and February 3, 2023 (drought and cold season). Samples were recognized by Dr. Hassan Ibrahim at the Biological Sciences Department, Faculty of Science, Sana'a University. Three plant specimens (BHSS 1805, BHSS 1806, and BHSS 1807) have been issued herbarium voucher numbers and deposited in the Herbarium of the Faculty of Science as a future reference. For chemical, antioxidant, and anti-microbial investigations, fresh leaves were properly cleaned with tap water and the gel was separated from the leaf skin. Furthermore, the leaf skin was dried in an air-circulating oven at 50°C until there were no further changes in leaf skin weight. Dried leaf skin was chopped and ground into a powder by a blender; powdered leaf skin and leaf gel were stored in an airtight cellophane container and kept in the refrigerator as a stock sample until needed (Hart and Fisher, 1971).

(a) (b)

Fig.1. *Aloe rubroviolacea* Schweinf, **a:** General view and habitat, **b:** Rhodoxanthin pigment from the leaf.

Methanol and aqueous (distilled water) leaf extracts were prepared independently; by employing the methodology Illustrated by Ibrahim *et al.* (2022); 30g of dried leaf skin and gel (separately) was extracted by 300 ml of methanol and distilled water (individually) in a conical flask, cotton-plugged, and processed by a rotary shaker at 190-220 rpm for 24 hours. The extracts were filtered using Whatman filter paper, the supernatant of each extract was collected, then solvents were evaporated to yield the final amount of *A. rubroviolacea* methanol (leaf skin and gel) and aqueous (leaf skin and gel) crude extracts individually. Extracts were maintained separately in a sealed container at 4°C for future studies (Chala and Yadessa, 2016; Ibrahim *et al.,* 2022**)**.

Phytochemical Screening

According to Venkatesan et al. (2009) and Ibrahim et al. (2022), the following assays were performed on *A. rubroviolacea* extracts to detect the presence of Alkaloids, Saponins, Phenol/Tannins, Steroids, Reducing sugars, Flavonoids, and Carbonyls:

Detection of Alkaloids

Weigh about 0.2 g. from each crude extract in a different test tube and warm it for 2 minutes with 2% Sulphuric acid, then filter into a separate test tube, add a few drops of Dragencloffs reagent, the presence of orange-red precipitates reveal the presence of alkaloids.

Detection of Saponins

Weigh about 0.2 g from each crude extract in an independent test tube, add 5 ml of distilled water, and heat to boil. The formation of small bubbles (foam) exhibits the presence of Saponin.

Detection of Phenol / Tannins

Mix a small quantity of each crude extract (individually) with distilled water in a test tube, and heat in a water bath. The mixture was filtered and ferric chloride was added to the filtrate. The attendance of tannins/phenols is indicated by the presence of dark green solutions.

Detection of Steroids

Add 2 mg of each crude extract (separately) to acetic anhydride, the mixture is heated till

boiling. The presence of Steroids is shown by the change in color of the mixture from violet to blue or green after adding 1 ml of concentrated sulfuric acid.

Detection of Reducing sugars

2 ml of each crude extract (individually) is added to 5 ml of distilled water, mixed then filtered. The filtrate was boiled for 2 minutes with 3-4 drops of Fehling's solutions A and B. The formation of an orange-red precipitate indicates the presence of reducing sugars.

Detection of Flavonoids

0.2 g. of each crude extract is dissolved (independently) in diluted Sodium hydroxide in a separate test tube then diluted Hydrochloride is added to the mixture. The occurrence of yellow solutions that turn colorless indicates the presence of flavonoids.

Detection of Carbonyl

The appearance of yellow crystals after adding 0.2g. of each crude extract (individually) to 2 ml of 2,4-Dinitrophenylhydrazine indicates the presence of Carbonyl.

Determination of functional groups by Fourier transform infrared (FTIR) assay

The functional groups of methanol (leaf skin extract and gel extract) and aqueous (leaf skin extract and gel extract) extracts were determined by IR spectra. Employing a mortar and pestle, a fraction of the powdered crude extract was combined with Potassium Bromide (KBr) salt (individually). The powder was compacted into a thin pellet by using a KBr press. Pellets were put in Perkin-Elmer FT-IR spectrometer 410 compartment and scanned throughout the IR range between 4000 and 400 cm^{-1} (Chen *et al*., 2008).

Determination of Plant Antioxidant capacity by using 1,1-Diphenyl-2-picrylhydrazyl free radical scavenging activity (DPPH) assay

The antioxidant activity of the *A. rubroviolacea* leaf skin (methanol and aqueous) extracts and gel (methanol and aqueous) extracts were determined by 1,1-Diphenyl-2-picrylhydrazyl free radical scavenging activity (DPPH) assay (modified after Benzie and Strain 1996; Al-Naqeb, 2015) in this assay the ascorbic acid was used as a standard antioxidant agent and DPPH solution (50 mg DPPH/100 ml of methanol) as a control

About 0.5 ml of a methanolic solution of DPPH was added to different concentrations (0.5, 1, 1.25, 1.5, 2 & 2.5 µg/ml) of ascorbic acid and different concentrations (2.5, 5, 7 &10 µg/ml) of *A. rubroviolacea* leaf skin (methanol and aqueous) extracts and gel (methanol and aqueous) extracts individually (ascorbic acid and all extracts were dissolved in methanol). Control and samples treated with DPPH solution (ascorbic acid and both extracts) were incubated in the dark at room temperature for 30 min, then the absorbance was recorded at 517 nm by Genova Life Science Analyser - Protein.

The radical scavenging effect was calculated in percentage by using the following formula: Radical scavenging effect (%) = (Ac – As)/Ac × 100, where Ac = absorbance of the control and As= absorbance of the tested (ascorbic acid and all extracts) sample (Al-Naqeb, 2015).

Tested microorganisms

The antimicrobial activity of *A. rubroviolacea* leaf skin (methanol and aqueous) and gel (methanol and aqueous) extracts were tested against four human pathogenic bacteria (*Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, and *Pseudomonas aeruginosa*) and one pathogenic fungus (*Candida albicans*). All microbial isolates were obtained from the National Central of Public Health Laboratory (NCPHL) -Sana'a.

Antibacterial bioassay

The efficacy of *A. rubroviolacea* methanol and aqueous extracts (leaf skin and gel) against bacteria was tested on Mueller Hinton Agar (MHA) medium by using the agar well-diffusion method (Iqbal *et al.,* 2015; Hussain et al., 2016; Shahzad *et al.,* 2017). For each bacterial pathogen, nine Petri dishes containing MHA were prepared (two testing plates for each

extract and one positive control plate). Each petri dish included 106 CFU/ml of the tested bacteria, which were cultured by spreading it over the surface of the medium with a sterile swab. A cork borer was used to make five to four wells (5 mm in diameter) in the agar medium (one in the middle, containing DMSO (negative control) and four to three wells at the edges of the petri dish containing different concentrations of the extract). To determine the antibacterial activity of *A. rubroviolacea* leaf skin (methanol and aqueous) extracts and gel (methanol and aqueous) extracts individually; a stock solution of *A. rubroviolacea* leaf skin (methanol and aqueous) extracts and gel (methanol and aqueous) extracts with a concentration of 100% was prepared (separately) by dissolving 10g of *A. rubroviolacea* leaf skin extracts and gel (methanol and aqueous) extracts (independently) in 10ml of DMSO. A serial dilution was prepared; 50, 25, 12.5, 6.25, and 3.12 % from the stock solution of each extract by using DMSO. 50 µl of each concentration (100; 50; 25, 12.5, 6.25, and 3.12 %) from each extract were pipetted into a well, while 50 µl of DMSO served as a negative control. However; a petri dish that contains Ciprofloxacin, Ampicillin, and Gentamicin antibiotics was used as a positive control plate. Plates were incubated at 37°C for 24 hours before measuring the inhibitory zone for each concentration. This process was repeated three times, and the inhibition zone diameter (mm) results were obtained as a mean. Furthermore, the Minimum Inhibitory Concentration (MIC) of each extract against the four chosen human pathogenic bacteria (*S. aureus*, *S. pneumonia*, *E. coli*, and *P. aeruginosa*) was taken (Mahesh, 2011; Ibrahim *et al.*, 2022).

Antifungal bioassay

The antifungal activity of *A. rubroviolacea* leaf skin (methanol and aqueous) extracts and gel (methanol and aqueous) extracts against *C. albicans* was determined using the agar well-diffusion method on Sabouraud Dextrose Agar (SDA) (Kalim *et al.,* 2016). Nine Petri dishes containing SDA were prepared (two testing plates for each extract and one positive control plate). A sterile swab was used to spread 100 microliters of *C. albicans* suspension (105 CFU/ml) over the SDA in each plate. Five to four wells (5 mm in diameter) were made by a cork borer in the agar medium (one in the center, containing DMSO (negative control) and four to three wells at the edges of the plate containing different concentrations of the extract). To determine the antifungal activity of *A. rubroviolacea* leaf skin (methanol and aqueous) extracts and gel (methanol and aqueous) extracts individually; a stock solution of *A. rubroviolacea* leaf skin (methanol and aqueous) extracts and gel (methanol and aqueous) extracts with a concentration of 100% was prepared (separately) by dissolving 10g of *A. rubroviolacea* leaf skin extracts and gel (methanol and aqueous) extracts (independently) in 10ml of DMSO. A serial dilution was prepared; 50, 25, 12.5, 6.25, and 3.12 % from the stock solution of each extract by using DMSO. 50 µl of each concentration (100; 50; 25, 12.5, 6.25, and 3.12%) from each extract were pipetted into a well, while 50 µl of DMSO served as a negative control. Furthermore; a petri dish that contains, Nystatin and Ketoconazole antibiotics was applied as a positive control plate. Plates were incubated at 37°C for 24 hours before measuring the inhibitory zone for each concentration. This process was repeated three times, and the inhibition zone diameter (mm) results were obtained as a mean. Furthermore, the Minimum Inhibitory Concentration (MIC) of each extract against *C. albicans* was taken (Mahesh, 2011; Ibrahim *et al.*, 2022).

RESULTS

Phytochemical Screening

Phytochemical screening in Table 1 shows that the leaf skin and gel extracts *of A. rubroviolacea* contain active pharmacological components. Based on the Phytochemical investigation; Alkaloids, Saponins, Tannins, Phenol, Steroids, Reducing sugar, Flavonoids, and Carbonly were detected in *A. rubroviolacea* methanol leaf (skin and gel) extract. On the other hand; Alkaloids, Saponins, Tannins, Reducing sugar, Flavonoids,

and Carbonly, were recorded in aqueous leaf skin extract; while Alkaloids, Saponins, Reducing sugar, Flavonoids, and Carbonly were found in aqueous gel extract.

Determination of function groups by Fourier transform infrared (FT-IR) assay:

Based on the FT-IR analysis, the function group in *A. rubroviolacea* methanol leaf skin and gel extracts are listed in Table 2. The peak at 3393.52cm^{-1} indicates the presence of the Hydroxyl group (OH) of medical compounds such as Phenols, Alcohol, Acids, and their derivatives in the methanol leaf skin (Figure 2) and methanol gel (Figure 3) extracts. The Peaks at the two wavelengths; 2924.85cm^{-1} and 2853.49cm^{-1} (Figure 2) and at the two wavelengths; 2921.96 cm^{-1} and 2853.49cm^{-1} (Figure 3) show the presence of methine function group (CH) of compounds in methanol leaf (skin and gel sequentially) extracts like Easters, Acids, and Alkanes. Week absorption and overtone/combination bands between wave lengths 2220.88cm^{-1} to 1922.90cm^{-1} (Figure 2) indicate the attendance of Aromatic rings in the methanol leaf skin extract while; the week absorption at wavelength 2220.56cm^{-1} (Figure 3) exhibit the presence of Aromatic rings in the methanol gel extract.

Table 1. Determination of chemical constituent in A. rubroviolacea leaf skin extracts and gel extracts.

Sr. No.	Phytochemical Components	Type of extract			
		Methanol leaf skin extract	Methanol gel extract	Aqueous leaf skin extract	Aqueous gel extract
1	Alkaloids	+ve	+ve	+ve	+ve
2	Saponins	+ve	+ve	+ve	+ve
3	Phenol + Tannins	+ve	+ve	+ve	-ve
4	Steroids	+ve	+ve	-ve	-ve
5	Reducing sugar	+ve	+ve	+ve	+ve
6	Flavonoids	+ve	+ve	+ve	+ve
7	Carbonyl	+ve	+ve	+ve	+ve

Table 2. FT-IR Spectrum of *A. rubroviolacea* leaf skin extracts and gel extracts.

Sr. No.	Function group	Methanol leaf skin extract	Methanol gel extract	Aqueous leaf skin extract	Aqueous gel extract
1	O-H	3393.52	3393.52	3420.52	3420.52
2	-CH	2924.85 & 2853.49	2921.96 & 2853.49	2922.9 & 2851.56	2920.03 & 2852.52
3	Overtone/ Combination – Aromatic rings	2220.88-1922.90	2220.56	Absent	Absent
4	C=C	1602.74 & 1457.12	1603.70	Absent	Absent
5	C=O	1700-1750	1700-1730	Absent	Absent
6	C-O	1226.89	1241.11	Absent	Absent
7	CH_3	Absent	Absent	Absent	1400-1300
8	Accumulate double bond / R - N =C =O R-N=C=S	Absent	2344.31-2313.46	Absent	Absent
9	-NH	Absent	Absent	Absent	1591.16
10	-CN	Absent	Absent	Absent	1287.40

Absorption at 1700-1750cm^{-1} in the case of methanol leaf skin extract (Figure 2) and at1700-1730 cm^{-1} in the case of methanol gel extract (Figure 3) indicates the attendance of carbonyl function group (C=O). Moreover; the absorption occurs in pairs at 1602.74 cm^{-1} and 1457.12cm^{-}

[1] exhibiting the presence of C=C in the methanol leaf skin extract (Figure 2); while the single peak at the wave length1603.70 cm^{-1} shows the attendance of C=C in the methanol gel extract (Figure 3).

The absorption peaks at the wavelength 1226.89cm^{-1} (Figure 2) and at wavelength 1241.11cm^{-1} (Figure 3) exhibit the presence of C-O in the methanol leaf skin extract and in the methanol gel extract correspondingly. Furthermore; the waves between 2344.31-2313.46 cm^{-1} indicate the presence of accumulated double bond / Isocyanates and Isothiocyanates in methanol gel extract (Figure 3).

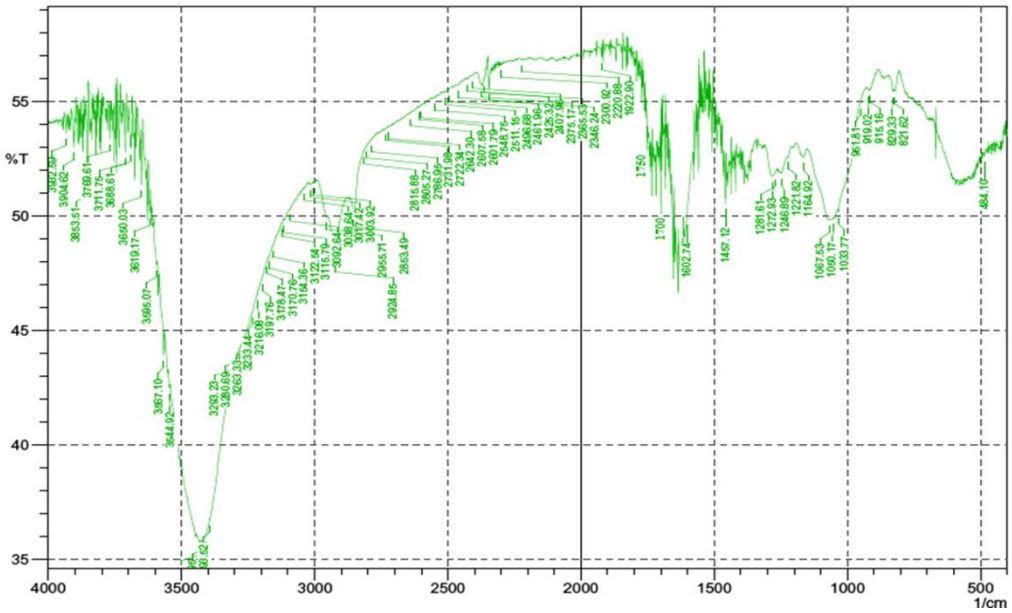

Fig. 2 . FT-IR spectrum analysis of *A. rubroviolacea* methanol leaf skin extract.

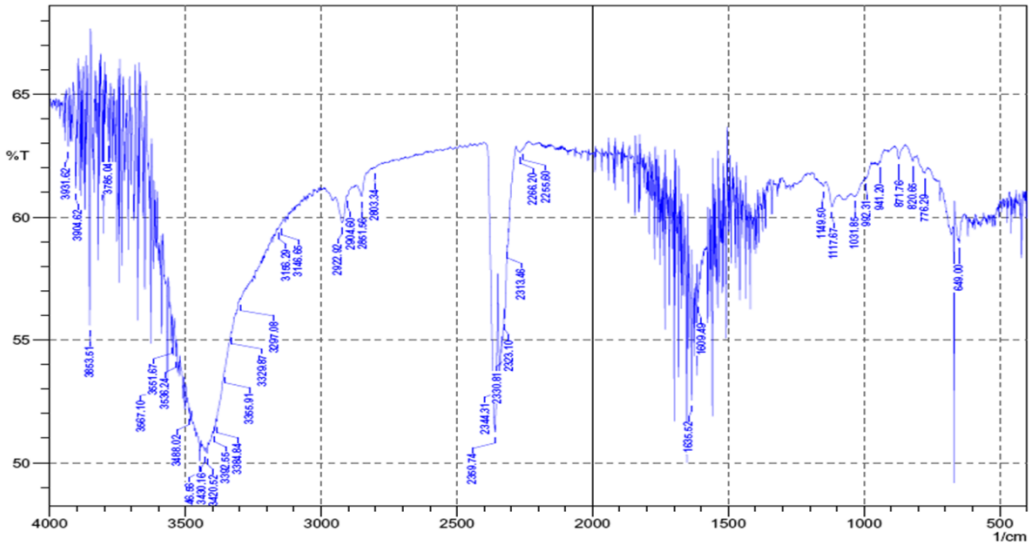

Fig. 3. FT-IR spectrum analysis of *A. rubroviolacea* methanol gel extract.

On the other hand, The FT-IR spectrum analysis of *A. rubroviolacea* aqueous (skin and gel, independently) extracts (Table 2, Figures 4 & 5) display the presence of Hydroxyl group (OH) in the aqueous leaf skin extract (3420.52 cm^{-1}) and in the aqueous gel extract (3420.52cm^{-1}). Moreover; the peaks at 2922.9 cm^{-1} & 2851.56 cm^{-1} indicate the presence of methine group (C-H) in the leaf skin aqueous extract (Figure 4), while; the two peaks at 2920.03 cm^{-1} and 2852.52 cm^{-1} exhibit the attendance of methine group (C-H) in the gel aqueous extract (Figure 5). However; the peaks at 1591.16cm^{-1}, 1400-1300cm^{-1}, and 1287.40cm^{-1} (Figure 5) show the presence of N-H, CH_3, and C-N, respectively in the aqueous gel extract.

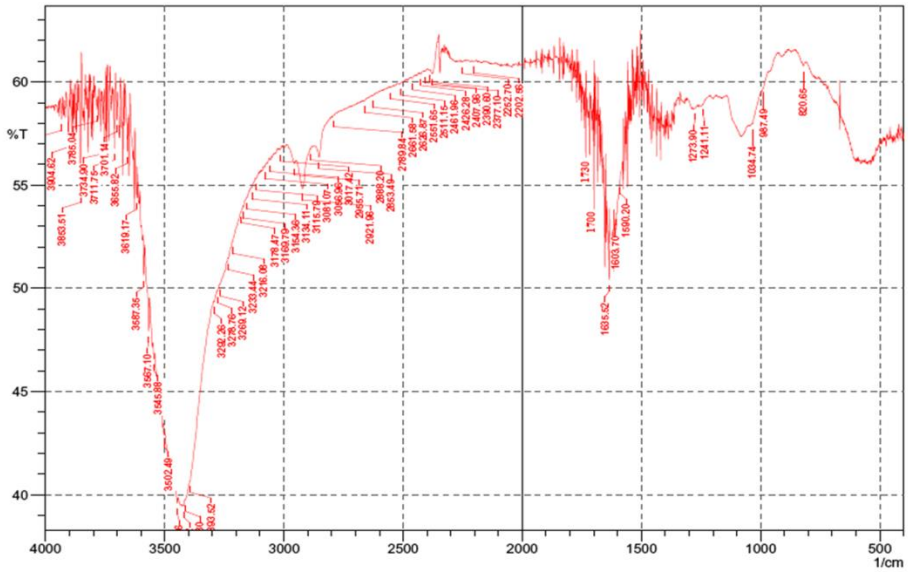

Fig. 4. FT-IR spectrum analysis of *A. rubroviolacea* aqueous leaf skin extract.

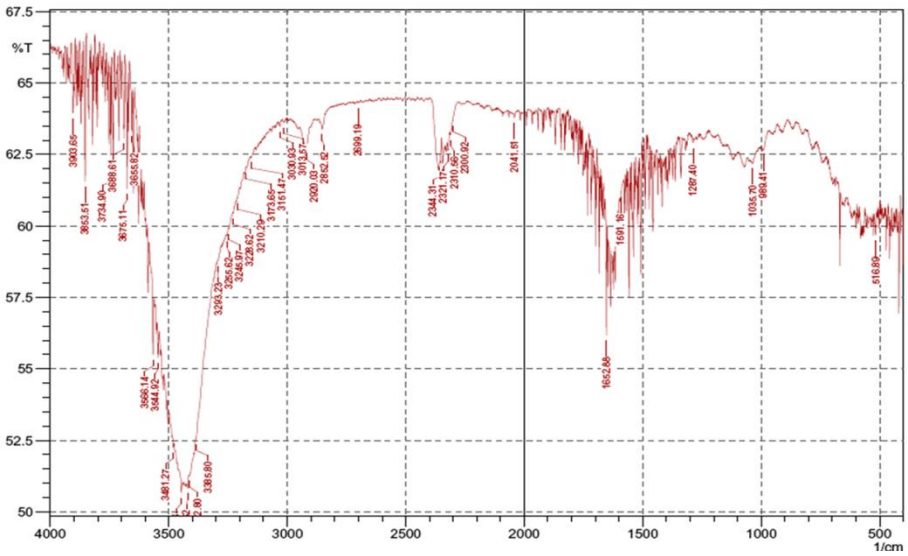

Fig. 5. FT-IR spectrum analysis of *A. rubroviolacea* aqueous gel extract.

Determination of Plant Antioxidant Activity by 1,1-Diphenyl-2-picrylhydrazyl free radical scavenging activity (DPPH) assay.

This method is based on the reduction of the stable free radical purple-colored DPPH in the presence of an antioxidant to the non-radical form of yellow-colored diphenypicrylhydrazyl DPPH-H. Figures 6, 7, 8 and 9 summarize the free radical scavenging activity of the *A. rubroviolacea* leaf skin and gel (methanolic and aqueous) extracts. It is clear that the methanol leaf skin extract exhibited higher antiradical activity towards DPPH radical; 20.5 %, 38.1 %, 54.6 %, and 72.2 % at concentrations; 2.5 µg/ml, 5µg/ml, 7.5µg/ml, and 10µg/ml (Figure 6) respectively; followed by aqueous gel (Figure 9) extract antiradical activity (21.2%, 31.7%, 42.8% & 52.9% at concentrations; 2.5 µg/ml, 5µg/ml, 7.5µg/ml, and 10µg/ml sequentially), and aqueous leaf skin (Figure 8) extract antiradical activity (12.6%, 24.4%, 35.2% and 41.9% at concentrations; 2.5 µg/ml, 5µg/ml, 7.5µg/ml, and 10µg/ml, serially). However, methanol gel (Figure 7) extract showed (15.3%, 20.6%, 26.5%, and 29.9% at concentrations; 2.5 µg/ml, 5µg/ml, 7.5µg/ml, and 10µg/ml sequentially) the lowest antiradical activity.

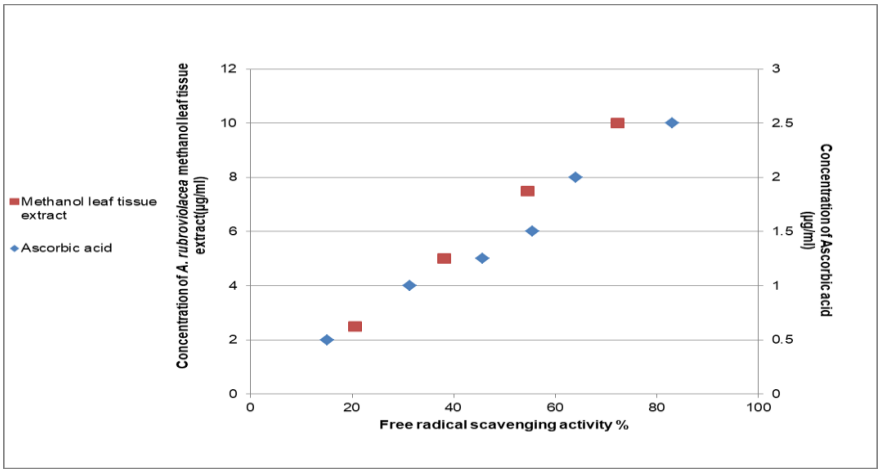

Fig. 6. Antioxidant activity of *A. rubroviolacea* methanol leaf skin extract and Ascorbic acid.

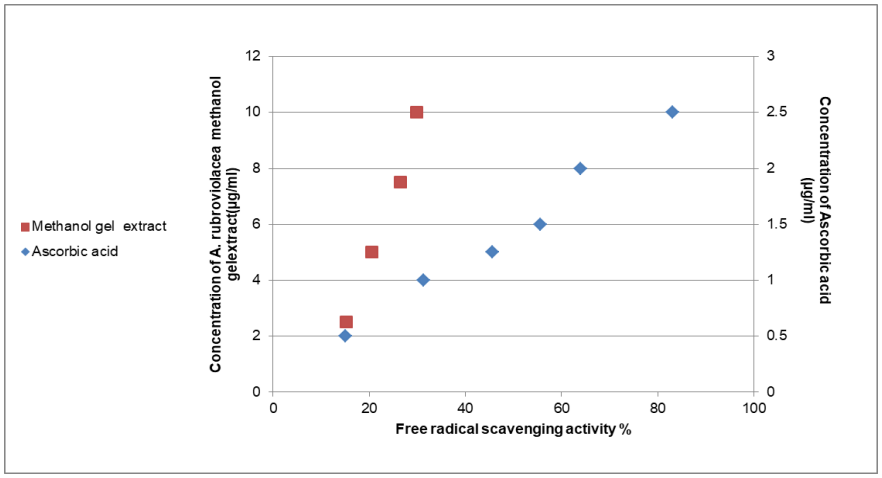

Fig. 7. Antioxidant activity of *A. rubroviolacea* methanol gel extract and Ascorbic acid.

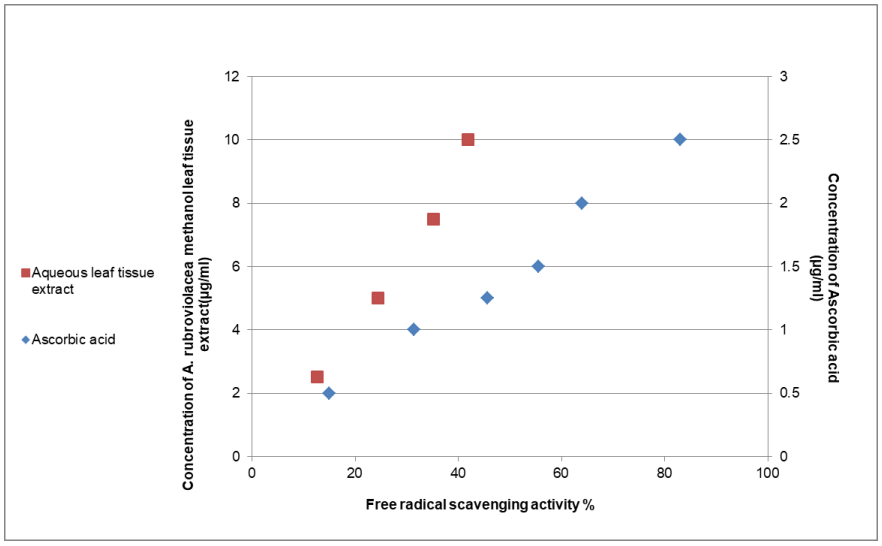

Fig. 8. Antioxidant activity of *A. rubroviolacea* aqueous leaf skin extract and Ascorbic acid.

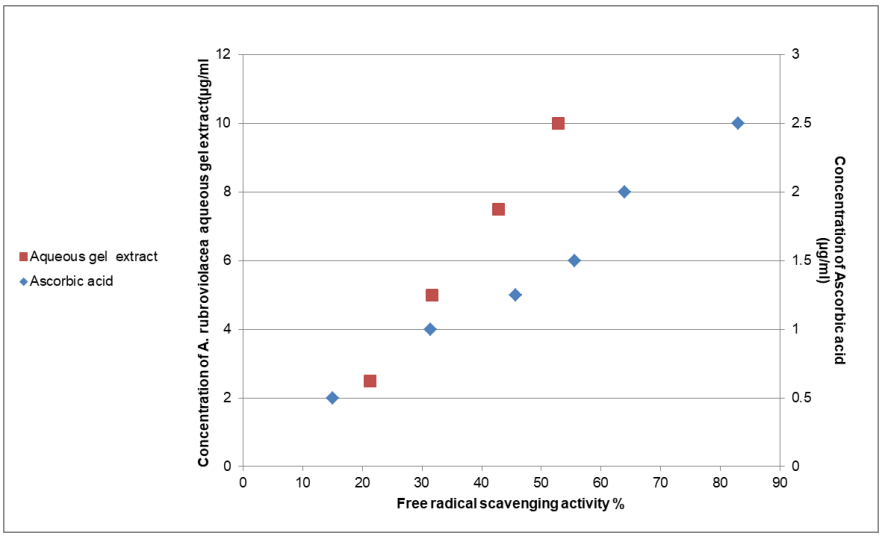

Fig. 9. Antioxidant activity of *A. rubroviolacea* aqueous gel extract and Ascorbic acid.

Antimicrobial Bioassay

Based on Table 3, *A. rubroviolacea* methanol leaf skin extract shows antibacterial activities against *S. aurous* (with a mean inhibition zone 10, 12, 14, 15, 18, and 20mm at concentration; of 3.12%, 6.25%, 12.5%, 25%, 50% and 100% respectively); *S. pneumoniae* (with a mean inhibition zone 10, 12and 14mm at concentration; of 25%; 50% and 100% correspondingly); *E .coli* (with a mean inhibition zone 10 and 13mm at concentration; of 50% and100% sequentially) and *P. aeruginosa* (with a mean inhibition zone 8, 9, 11 and 12mm at concentration; of 12.5%, 25%, 50% and 100% consecutively). *A. rubroviolacea* showed antifungal activity against *C. albicans* with a mean inhibition zone 10, 11, 12, 13, and 15 mm at concentrations; of 6.25%, 12.5%, 25%, 50%, and 100% respectively (Table 4).

Table 3. Inhibition zone diameters (mm) of A. rubroviolacea methanol leaf skin extract against tested bacterial pathogens.

Tested Bacterial Pathogens	Type	Extraction concentration							Control DMSO	Antibiotics			MIC (mm)
		100%	50%	25%	12.5%	6.25%	3.12%	1.5%		CIP (5µg)	AMP (10µg)	GEN (10µg)	
S. aurous	+ G	20	18	15	14	12	10	-	-	40	20	-	10
S. pneumoniae	+ G	14	12	10	-	-	-	-	-	10	19	-	10
E. coli	- G	13	10	-	-	-	-	-	-	-	-	-	10
P. aeruginosa	- G	12	11	9	8	-	-	-	-	30	-	14	8

+G= Gram positive; -G= Gram negative; Antibiotics: CIP= Ciprofloxacin; AMP = Ampicillin; GEN = Gentamicin.

Table 4. Inhibition zone diameters (mm) of A. rubroviolacea methanol leaf skin extract against tested fungal pathogen.

Tested Fungal Pathogen	Extraction concentration							Control DMSO	Antibiotics		MIC (mm)
	100%	50%	25%	12.5%	6.25%	3.12%	1.5%		NS (50µg)	KT (30µg)	
C.albicans	15	13	12	11	10	-	-	-	20	-	10

Antibiotics: NS= Nystatin & KT= Ketoconazole.

A. rubroviolacea methanol gel extract displayed antimicrobial activities against *S. aurous* (with a mean inhibition zone of 10, 12, 13, 14, 16 and 19 mm at concentrations; of 3.12%, 6.25%, 12.5%, 25%, 50% and 100% correspondingly), *E. coli* (with a mean inhibition zone 11 and 13mm at concentrations; of 50% and 100% respectively), *P. aeruginosa* (with a mean inhibition zone11, 13 and 15 mm at concentrations; of 25%; 50% and100% serially) (Table 5) and *C. albicans* (with a mean inhibition zone 20, 22, 24, 26, 28, 32 and 36 mm at concentrations; of 1.5%, 3.12%, 6.25%, 12.5%, 25%, 50% and100% sequentially) (Table 6).

Table 5. Inhibition zone diameters (mm) of A. rubroviolacea methanol gel extract against tested bacterial pathogens.

Tested Bacterial Pathogens	Type	Extraction concentration							Control DMSO	Antibiotics			MIC (mm)
		100%	50%	25%	12.5%	6.25%	3.12%	1.5%		CIP (5µg)	AMP (10µg)	GEN (10µg)	
S. aurous	+ G	19	16	14	13	12	10	-	-	40	20	-	10
S. pneumoniae	+ G	-	-	-	-	-	-	-	-	10	19	-	-
E. coli	- G	13	11	-	-	-	-	-	-	-	-	-	11
P. aeruginosa	- G	15	13	11	-	-	-	-	-	30	-	14	11

+G= Gram positive; -G= Gram negative; Antibiotics: CIP= Ciprofloxacin; AMP = Ampicillin; GEN = Gentamicin.

Table 6. Inhibition zone diameters (mm) of A. rubroviolacea methanol gel extract against tested fungal pathogen.

Tested Fungal Pathogen	Extraction concentration							Control DMSO	Antibiotics		MIC (mm)
	100%	50%	25%	12.5%	6.25%	3.12%	1.5%		NS (50µg)	KT (30µg)	
C.albicans	36	32	28	26	24	22	20	-	25	-	20

Antibiotics: NS= Nystatin & KT= Ketoconazole.

The aqueous leaf skin extract of *A. rubroviolacea* showed antimicrobial activity against *S. aurous* (with a mean inhibition zone10, 12, 14and 16 mm at concentrations; of 12.5%, 25%, 50% and100% correspondingly), *E. coli* (with a mean inhibition zone 9, 11, 18,

and 19 mm at concentrations; of 12.5%, 25%, 50% and100% respectively*),* *P. aeruginosa* (with a mean inhibition zone 9, 11 and 14 mm at concentrations; of 25%, 50% and100% serially) (Table 7) and *C. albicans* (with a mean inhibition zone 20, 24, 28 and 30mm at concentrations; of 12.5%, 25%, 50% and100% sequentially) (Table 8).

Table 7. Inhibition zone diameters (mm) of A. rubroviolacea aqueous leaf skin extract against tested bacterial pathogens.

Tested Bacterial Pathogens	Type	Extraction concentration							Control DMSO	Antibiotics			MIC (mm)
		100%	50%	25%	12.5%	6.25%	3.12%	1.5%		CIP (5µg)	AMP (10µg)	GEN (10µg)	
S. aurous	+ G	16	14	12	10	-	-	-	-	40	20	-	10
S. pneumoniae	+ G	-	-	-	-	-	-	-	-	10	19	-	-
E. coli	- G	19	18	11	9	-	-	-	-	-	-	-	9
P. aeruginosa	- G	14	11	9	-	-	-	-	-	30	-	14	9

+G= Gram positive; -G= Gram negative; Antibiotics: CIP= Ciprofloxacin; AMP = Ampicillin; GEN = Gentamicin.

Table 8. Inhibition zone diameters (mm) of A. rubroviolacea aqueous leaf skin extract against tested fungal pathogen.

Tested Fungal Pathogen	Extraction concentration							Control DMSO	Antibiotics		MIC (mm)
	100%	50%	25%	12.5%	6.25%	3.12%	1.5%		NS (50µg)	KT (30µg)	
C.albicans	30	28	24	20	-	-	-	-	25	-	20

Antibiotics: NS= Nystatin & KT= Ketoconazole.

Furthermore; *A. rubroviolacea* aqueous gel extract inhibited the growth of *S. aurous* (with a mean inhibition zone10, 11, and 13 mm at concentrations; of 25%, 50% and100% correspondingly), *E. coli* (with a mean inhibition zone 11 and 13 mm at concentrations; of 50% and100% respectively), *P. aeruginosa* (with a mean inhibition zone 10, 11, 12 and 13 mm at concentrations; of 12.5%, 25%, 50% and100% serially) (Table 9) and *C. albicans* (with a mean inhibition zone 12, 16, 20, 27, 30 and 35mm at concentrations; of 3.12%, 6.25%, 12.5%; 25%; 50% and100% sequentially) (Table 10).

On the other hand, *A. rubroviolacea* methanol gel extract and aqueous (leaf skin and gel) extracts did not inhibit the growth of *S. pneumonia*.

The Minimum inhibition concentration (3.12%) of the *A. rubroviolacea* methanol (leaf skin and gel) extracts showed the highest antibacterial activity (10 mm) against *S. aurous*, in competing with the Minimum inhibition concentration (MIC) of aqueous (leaf skin and gel) extracts. Moreover; the Minimum inhibition concentration (12.5%) of *A. rubroviolacea* aqueous leaf skin extract exhibits the highest antibacterial activity (9 mm) against *E. coli*, in competition with the Minimum inhibition concentration (MIC) of the other extracts. Furthermore, the MIC of the aqueous gel extract (12.5%) shows the highest antibacterial activity (10 mm) against *P. aeruginosa*, in competition with the MIC of the other extracts. On the other hand, the MIC of the methanol gel extract (1.5%) displays the highest antifungal activity (20mm) against *C. albicans.*

Table 9. Inhibition zone diameters (mm) of A. rubroviolacea aqueous gel extract against tested bacterial pathogens.

Tested Bacterial Pathogens	Type	Extraction concentration							Control DMSO	Antibiotics			MIC (mm)
		100%	50%	25%	12.5%	6.25%	3.12%	1.5%		CIP (5µg)	AMP (10µg)	GEN (10µg)	
S. aurous	+ G	13	11	10	-	-	-	-	-	40	20	-	10
S. pneumoniae	+ G	-	-	-	-	-	-	-	-	10	19	-	-
E. coli	- G	13	11	-	-	-	-	-	-	-	-	-	11
P. aeruginosa	- G	13	12	11	10	-	-	-	-	30	-	14	10

+G= Gram positive; -G= Gram negative; Antibiotics: CIP= Ciprofloxacin; AMP = Ampicillin; GEN = Gentamicin.

Table 10. Inhibition zone diameters (mm) of A. rubroviolacea aqueous gel extract against tested fungal pathogen.

Tested Fungal Pathogen	Extraction concentration							Control DMSO	Antibiotics		MIC (mm)
	100%	50%	25%	12.5%	6.25%	3.12%	1.5%		NS (50µg)	KT (30µg)	
C.albicans	35	30	27	20	16	12	-	-	25	-	10

Antibiotics: NS= Nystatin & KT= Ketoconazole.

Moreover; our results showed that the chosen antibiotics did not exhibit any antibacterial action against *E. coli*, whereas *A. rubroviolacea* methanol extracts (leaf skin and gel separately) extracts and aqueous (leaf skin and gel individually) showed antibacterial activity against *E. coli* in 2 (50% and 100% with an inhibition zone 10 & 13 mm sequentially), 2 (50% & 100% with an inhibition zone 11 & 13 mm correspondingly), 4 (12.5%, 25%, 50% &100%with an inhibition zone 9, 11, 18 & 19 mm respectively), 2 (50% & 100% with an inhibition zone 11 & 13 mm sequentially) concentrations consecutively.

DISCUSSION

The Phytochemical results are comparable with the findings of Das and Srivastav (2015) where they mentioned that *A. vera* methanol leaf extract in general, contains tannins, saponins, flavonoids, and terpenoids, and with the outcomes of Ibrahim et al. (2022) where they cited that *A. fleurentinorum* methanol leaf (skin and gel individually) extracts comprise; Alkaloids, Saponins, Phenols, Tannins, Reducing sugar, Flavonoids, and Carbonyl, while; steroids were presented only in methanol gel extract. Moreover, the Phytochemical analysis of aqueous leaves skin and gel extract is compatible with the findings of earlier studies where they cited that *A. vera* aqueous leaf extract contains; tannins, saponins, flavonoids & terpenoids (Raad *et al.*, 2021); while Ibrahim et al. (2022) mentioned that *A. fleurentinorum* aqueous leaf (skin and gel individually) extracts comprise; Alkaloids Saponins, Flavonoids, and Carbonyl while; Reducing sugar was only presented in gel aqueous extract.

Moreover; the FT-IR spectrum analysis results of *A. rubroviolacea* methanol (leaf skin & gel individually) and aqueous (leaf skin & gel separately) extracts are closely similar to the findings of Kassama *et al.* (2015); Abbasi *et al.* (2020); Qasem (2021); Rani et al. (2021) and Ibrahim et al. (2022). The content of antioxidant agents such as; phenolic compounds, and carotenoids (xanthophyll) in plant raw materials enables them to become a source of effective and safe natural antioxidants (Hęś, *et al.*, 2019). Therefore the antioxidant capacity of the *A. rubroviolacea* leaf skin and gel (methanol and aqueous) extracts may be due to the presence of Polyphenols (Flavinodas and Tannins), Phenols, and Rhodoxanthin which is a xanthophyll red pigment, induced by drought, and it has a stronger antioxidant capacity compared to other carotenoids, such as β-carotene (Merzlyak *et al.*, 2005; Mussagy *et al.*, 2023).

However; the low antioxidant activity of methanol gel extract is may due to the presence of accumulated double bond / Isocyanates and Isothiocyanates which can reduce the radical scavenging activity of the extraction. Furthermore; the antioxidant activities of *A. rubroviolacea* extracts are close to the findings of Cho et al. (2014) where they cited that *A. vera* skin 70% ethanol extract shows the highest DPPH radical scavenging activity (55%) when compared with *A. vera* skin-water extract, *A. vera* gel-70% ethanol extract, and *A. vera* gel-water extract. Moreover, the antioxidant activities of *A. rubroviolacea* gel (methanol and aqueous) extracts are compatible with Gorsi et al. (2019) where they concluded that the presence of Polyphenols (Flavinodas) and phenols in *A. vera* gel, powder is the reason for the antioxidant capacity of *Aloe* (*A. vera*) gel powder, since Polyphenols (Flavinodas) and phenols are a group of compounds containing phenolic hydroxyl attached to ring structures, due to which they function as an antioxidant.

On the other hand, the antimicrobial activities of *A. rubroviolacea* methanol (leaf skin & gel) and aqueous (leaf skin & gel) extracts are comparable with the outcomes of Agarry et al. (2005), Lawrence et al. (2009), Stanley et al. (2014), Sandeep et al. (2015), and Ibrahim et al. (2022). The antimicrobial activity of *A. rubroviolacea* extracts is may due to the presence of Polyphenols (tannins and flavonoids), phenols, and rhodoxanthin pigment which are a group of substances that act as antioxidants because they contain phenolic hydroxyl and their mechanism of action is related to their ability to donate hydrogen and scavenging free radicals (Merzlyak *et al.,* 2005; Gorsi *et al.*, 2019; Mussagy *et al.*, 2023).

The ability to scavenge the free radicals allows these compounds to interact with reactive oxygen species (ROS) which can lead to oxidative stress and damage the microbial cells (Oliveira *et al.*, 2016). The unevenly antioxidant capacity and antimicrobial activities of *A. rubroviolacea* leaf skin and gel (individually) extracts against the selected bacteria and fungi are caused by the unequal presence of phenols, tannins, flavonoids, and rhodoxanthin pigment (which dissolves partially in water) in the extracts.

CONCLUSION

The Phytochemical screening and FT-IR spectrum analysis exhibit the presence of some medical compounds especially flavonoids and phenolics in the methanol extracts (leaf skin extract and gel extract) However; the aqueous leaf skin extract contains flavonoids and tannins, while the aqueous gel extract contains flavonoids. Moreover; The methanol leaf skin extract exhibited a high antioxidant activity followed by aqueous gel extract and leaf skin extract; while the methanol gel extract showed the lowest antiradical activity, this may due to the presence of accumulated double bond / Isocyanates and Isothiocyanates which can reduce the radical scavenging activity of the extraction because of there less stable.

The previous results show that the methanol leaf skin extract exhibits the highest antimicrobial activity against the selected microbial pathogens followed by the methanol gel extract. This could be related to the presence of phenolics, flavonoids & tannins compounds, and rhodoxanthin pigment in the methanol leaf skin extract which has the highest antioxidant activity.

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CONFLICT OF INTEREST

Authors hereby declare that they have no conflict of interest.

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