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Fourier-transform Infrared (FTIR) Characterization and Radical Scavenging Activity *Ziziphus spina-christi* (Rhamnaceae) Rootbark Fractions

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ABSTRACT

This study focused on the secondary metabolite characterization and radical scavenging activity of the chloroform (CF), ethyl acetate (EF), and aqueous fractions (AF) of *Ziziphus spina-christi* to ascertain its therapeutic potential against oxidative stress. Fourier-transform infrared (FTIR) characterization and determination of *in vitro* radical scavenging activity of the plant were carried out. Alkaloids, saponins, and flavonoids were present in all the fractions with steroids absent in the AF. The FTIR characterization detected alcohol, conjugated alkenes, and amine groups in the CF and EF. However, alkanes, aromatic amines, sulfonates, and monosubstituted alkanes were also detected in the latter. Moreover, carboxylic acid, alkane, alkene, amines, and phenols were identified in the AF. The EF (72.46 ± 0.55 µg/ml AAE) and AF (71.51 ± 0.46 µg/ml AAE) demonstrated a significantly ($p < 0.05$) higher total antioxidant capacity (TAC) than CF (50.33 ± 0.27 µg/ml AAE). The AF (54.07 ± 0.97 µg/ml AAE) exhibited a significantly ($p < 0.05$) higher total reducing power (TRP) than the EF (42.76 ± 1.60 µg/ml AAE) and CF (30.13 ± 1.32 µg/ml AAE). A significantly ($p < 0.05$) higher percentage of lipid peroxidation inhibition was exhibited by the CF (71.25% ± 3.41) compared to the EF (54.17% ± 2.66). Moreover, all the fractions showed significantly ($p < 0.05$) higher inhibition than ascorbic acid (18.33% ± 1.56). The CF (0.16 ± 0.01 nmol/ml) and EF (0.21 ± 0.01 nmol/ml) demonstrated a significantly ($p < 0.05$) lower MDA concentration than the AF (0.42 ± 0.01 nmol/ml) and ascorbic acid (0.38 ± 0.02 nmol/ml). Conclusively, the *Z. spina* rootbark has potential antioxidant application in oxidative stress therapy with a focus on anti-lipid peroxidation for the CF though the AF has better TAC and TRP.

Keywords: Antioxidants; Antioxidant activity; *in vitro* activity; Lipid peroxidation; Malonaldehyde concentration; Oxidative stress

INTRODUCTION

Oxidative stress is a state of redox imbalance characterized by increased free radical generation, including reactive oxygen species (ROS) and reactive nitrogen species (RNS) with an accompanied depletion in the inherent antioxidant system of the body, leading to inflammation and mitochondria and DNA damage (Kıran et al., 2023). Increased oxidative stress exacerbates disease conditions and is often identified by biomarkers including serum malondialdehyde and promotes disease onset (Zeliger, 2023). In renal pathology, oxidative stress leads to disturbance in function *via* renal fibrosis, ischemia-reperfusion injury, and destruction of the structural components of the kidney (Saini, 2022). In cardiovascular ailment, the onset of oxidative stress is linked to a reduction in nitric oxide bioavailability and inflammation, contributing to endothelial dysfunction (Shaito et al., 2022). Additionally, this condition is linked to atherosclerosis, ischemia-reperfusion injury, and heart failure, affecting cellular components and leading to cell death (Wang & Kang, 2020). Moreover, inflammation is exacerbated by oxidative stress in airway ailments, depleting the antioxidant system and promoting asthma and lung cancer (Mozzini & Pagani, 2022). Oxidative stress is primarily triggered by persistent hyperglycemia in diabetes, resulting in micro- and macro-vascular complications (Chikezie et al., 2015). Additionally, diabetic complications, including neuropathy, nephropathy, and retinopathy are linked to oxidative stress (Chikezie et al., 2015). Although oxidative stress is a vital component in several diseases, antioxidants play a crucial counter role in the therapy of these diseases.

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Antioxidants donate electrons to counteract the harmful effects of oxidative stress by neutralizing the free radicals and preventing their accompanied damage to cells (Whayne et al., 2016; Rammohan et al., 2023). These compounds act by scavenging free radicals in addition to preventing their formation, activating enzymes, chelating metals, regulating gene transcription, and inhibiting oxidases (Barreca, 2020; Sundaram Sanjay & Shukla, 2021; Miftode, 2022). Furthermore, they modulate the redox signaling pathways and generate and enhance the antioxidant activities of bioactive metabolites (Hunyadi, 2019). These compounds directly act by hydrogen atom transfer, proton-coupled electron transfer, and radical adduct formation (Markovic et al., 2019; Ivanova et al., 2020). Moreover, they act indirectly *via* mitochondrial modulation and hormesis, promoting detoxification and vitagenes (Franco et al., 2019).

There are several inherent antioxidant systems in the body, including glutathione, superoxide dismutase, and catalase which often depleted in a state of oxidative stress (Birben et al., 2012). Therefore, a need for an exogenous supply for augmentation. Exogenous antioxidants from food and medicinal plants are regarded as alternate remedies in oxidative stress therapy (Rahaman et al., 2023; Rammohan et al., 2023). Antioxidant mitigates the aging process by downregulating the inflammatory genes and pathways (Türkoğlu et al., 2023). The antioxidant properties of medicinal plants are explored in the phytotherapy of several diseases, including cancer, diabetes, and cardiovascular ailments. Different techniques are employed to ascertain the antioxidant activities of these plants, including *in vitro* and *in vivo* techniques.

Medicinal plants present a reservoir of bioactive compounds of different efficacy with several pharmacological properties, such as antioxidant, antidiabetic, anti-inflammatory, and anticancer activities (Khan et al., 2019; Ahn & Park, 2020; Amraei & Ahmadi, 2022; Dahiru & Nadro, 2022b; Dahiru & Nadro, 2022a; Dahiru, 2023; Dahiru et al., 2023a; Dahiru et al., 2023b; Dahiru et al., 2024a; Dahiru & Musa, 2024). The antidiabetic activity of *H. thebaica* fruit was previously reported and attributed to its hypoglycemic effect and reduced level of diabetic biomarkers (Dahiru & Nadro, 2022a). The inhibition of oxidative stress-promoting enzymes by stigmasterol and 4,4-Dimethylcyclohex-2-en-1-ol identified in *Ximenia americana* was reported previously (Dahiru et al., 2022; Dahiru et al., 2024a). Similarly, several compounds previously identified in *Diospyros mespiliformis* exhibited antioxidant and anti-inflammatory activities (Dahiru & Musa, 2024). Several studies previously reported the antioxidant activities of different medicinal plants, attributed to their phytochemical components (Dahiru & Nadro, 2022b; Dahiru et al., 2023a; Dahiru et al., 2024a; Dahiru & Musa, 2024; Dahiru et al., 2024c). Dahiru & Musa (2024) reported the antioxidant and anti-inflammatory effects of *Diospyros mespiliformis via* free radical scavenging effects and mitigating the activities of inflammation- and oxidative stress-promoting enzymes. Additionally, the radical scavenging activities of *H. thebaica, Tamarindus Indica*, and *Corchorus olitorius via in vitro* techniques were reported previously (Dahiru & Nadro, 2022b; Dahiru et al., 2023a; Dahiru et al., 2024b).

Phytochemicals, otherwise called secondary metabolites are produced by plants for protective purposes (Kumar et al., 2023). These classes of compounds, such as saponins and flavonoids are linked to several pharmacological activities including antioxidant activities. Alfalfa saponins demonstrated antioxidant activities by elevating antioxidant enzyme levels and down-regulating malonaldehyde and lactate dehydrogenase in hydrogen peroxide-induced-oxidative stress (Cui et al., 2020). An apigenin derivative from *Selaginella doederleinii* exhibited a significant antioxidant potential with a promising anticancer effect (Muema et al., 2022). Quercetin, another flavonoid isolated from *Baccharis macrantha* leaves was previously linked to radical scavenging and anti-inflammatory activities of the plant (Rosero et al., 2022). The pharmacological activities of different flavonoids including their antioxidant activities are attributed to the substitution patterns of their C6-C3-C6 rings (Shen et al., 2022). In another study, 3-(azepan-1- yl)-1,2-benzothiazole 1,1-dioxide and caryophyllene oxide identified in *Diospyros mespiliformis* exhibited inhibitory potential against oxidative stress-promoting enzymes and linked to the radical scavenging activities of the plant (Dahiru & Musa, 2024).

Ziziphus spina-christi is a Rhamnaceae family species, originating from different parts of the world, including Africa, the Mediterranean, and Asia, commonly called Jujube (Saied et al., 2008). The plant is employed in the phytotherapy of inflammation in traditional medicine due to its antioxidant activities. Moreover, several *in vitro* and *in silico* studies reported the antioxidant activities of different parts of the plants linked to

the alleviation of different ailments. Furthermore, this has been previously reported as a valuable source of novel therapeutics, targeting different ailments including oxidative stress (Asgarpanah & Haghighat, 2012; Hussein, 2019). Thus, in our report, we characterized the phytoconstituents and determined the antioxidant activities of the chloroform, ethyl acetate, and aqueous fractions of *Z. spina-christi* rootbark (ZSRB) to further document the antioxidant activity of the plant.

MATERIALS AND METHODS

Plant material

Z. spina-christi (ZS) root was obtained from the Jimeta bypass of Yola North Local Government Area of Adamawa State. The sample was identified by a Forest Technologist from the Forestry Technology Department of Adamawa State Polytechnic, Yola.

Methods

Extraction and Fractionation

The root was washed with tap water to remove debris and sand, followed by removal of the bark, air-drying, and grinding to powder using mortar and pestle. Exactly one kilogram of the powder was extracted by maceration for 7 days in 70% (v/v) ethanol in an air-tight container subjected to daily agitation. A Whatman No.1 filter paper was used to filter the mixture and obtain the filtrate which was dried under reduced pressure using a rotary evaporator (Buchi Rotavapor R-200) to obtain the crude extract (140 g). The fractionation was carried out in a separating funnel *via* the solvent partition technique. Exactly one hundred grams of the crude extract was completely dissolved in 250 ml of distilled water and transferred to the separating funnel, followed by the addition of an equal volume of chloroform and vigorous shaking. The chloroform layer was collected and the aqueous layer was continuously washed with the chloroform until a clear chloroform layer was observed. The aqueous layer was further washed with ethyl acetate until a clear ethyl acetate layer was obtained. The chloroform, ethyl acetate, and aqueous layers were dried as mentioned for the crude extract to obtain 16.01, 7.28, and 21.50 g of chloroform (CF), ethyl acetate (EF), and aqueous (AF) fractions, respectively.

Secondary Metabolites Identification

The obtained fractions were subjected to preliminary screening to determine the presence of secondary metabolites. The presence of alkaloids, saponins, steroids, glycosides, terpenoids, and flavonoids was tested according to previously described standard methods (Evans, 2009).

Fourier-transform Infrared (FTIR) Characterization

The FTIR characterization was done as we described previously using the Perkin-Elmer FTIR (series 100) (Dahiru et al., 2024b). The procedure was as we previously described while the functional group identification was done by spectrum interpretation using the IR spectrum Table (Merck, 2023).

Radical Scavenging Activity

The radical scavenging activity was evaluated according to previously described methods for total antioxidant capacity (TAC), total reducing power (TRP), ferric thiocyanate (FTC), and thiobarbituric acid assay (TBA).

Total Antioxidant Capacity (TAC)

The method previously described by Prieto et al. (1999) was followed to determine the TAC of the samples. Exactly 0.5 ml of 300 µg/ml of distilled-water dissolved sample was mixed with the phosphomolybdate reagent and capped, followed by 10 minutes of 95 °C incubation. The absorbance of the sample was read at 695 nm after cooling against a blank (reagent without the sample). The ascorbic acid (AA) calibration curve was obtained from different concentrations (20-100 µg/ml) of AA subjected to the aforementioned sample treatment. All procedures were in triplicate. The TAC of the sample was expressed as AA equivalent (AAE) in µg/ml.

Total Reducing Power (TRP)

The protocol of Oyaizu (1986) was used to determine the TRP of the samples. Exactly 0.25 ml of 100 µg/ml of distilled-water dissolved sample was mixed with 0.625 ml of 0.2 M phosphate buffer (pH 6.6) and 0.625 ml of 1% (w/v) potassium ferrocyanide, followed by 20 minutes of 50 °C incubation. Exactly 0.625 ml of 10% (w/v) trichloroacetic acid (TCA) was added and centrifuged for 10 minutes at 3000 rpm. Furthermore, 1.8 ml of the upper layer was mixed with an equal volume of distilled water, and 0.36 ml of 0.1% $FeCl_3$ was added. The sample absorbance was read at 700 nm against a blank (reagents without sample). Moreover, the AA calibration curve was obtained from varying concentrations (20-100 µg/ml) of AA subjected to the same treatment as the sample. All procedures were in triplicate. The TRP of the sample was expressed as AA equivalent (AAE) in µg/ml.

Ferric Thiocyanate (FTC)

The anti-lipid peroxidation of the samples was determined according to the procedure previously described by Kikuzaki & Nakatani (1993) using FTC. Exactly 4 ml of 1 mg/ml absolute ethanol dissolved sample and sample were added to 0.05 M pH 7 phosphate buffer and 3.9 ml distilled water, followed by capping and 10 minutes 40 °C dark-oven incubation. Exactly, 0.1 ml of the solution was mixed with 30% (w/v) ammonium thiocyanate, 9.7 ml of 75% (v/v) ethanol, and 0.1 ml of 0.02 M ferrous chloride in 3.5% (v/v) HCl. The mixture without a sample was used as a control. The absorbance was read immediately and after every 24 hours at 532 nm until the maximum absorbance of the control was observed. The percentage inhibition was determined according to Equation 1. The procedure was carried out in triplicates.

$$\% \text{ Inhibition} = 100 - (\frac{\text{At}}{\text{Ac}} \text{ x } 100) \qquad \text{Equation 1}$$

Where At = Absorbance of sample while Ac = Absorbance of control

Thiobarbituric Acid Assay (TBA)

The anti-lipid peroxidation activity of the samples was further evaluated by the TBA method as previously described by Kikuzaki & Nakatani (1993). One ml of the prepared sample and AA in the FTC method were mixed with 2 ml of 20% (w/v) TCA and 2 ml of 0.5% (w/v) TBA, followed by 20 minutes of 90 °C water bath incubation. The mixture was centrifuged at 3000 rpm after cooling and the absorbance of the supernatant was read at 532 nm on the last of the FTC method. The extinction coefficient 156 mM^{-1} cm^{-1} was used to determine the malonaldehyde (MDA) concentration using Equation 2.

$$\text{MDA concentration} = (\frac{\text{OD}}{\text{EC}} \text{ x Sample volume}) \qquad \text{Equation 2}$$

Where OD = Absorbance of the sample while EC = Extinction coefficient

Statistics

All the procedures in our reports were carried out in triplicates and the data obtained was expressed as the mean of ± standard error of the mean of triplicate determinations (± SEM). The group difference was evaluated by One-way analysis of variance, followed by Tukey's multiple comparison test. The obtained data was evaluated using the Statistical Package for Social Sciences (SPSS) version 22 software.

RESULTS

Secondary Metabolites Identification

The identified secondary metabolites are presented in Table I. Alkaloids, saponins, and flavonoids were present in all the fractions while steroids were only in the CF and EF. Moreover, glycosides and terpenoids were absent in all of the fractions.

Table I. Secondary metabolites present in CH, HF, and AF of ZSRB

Secondary metabolite	CF	EF	AF
Alkaloids	+	+	+
Saponins	+	+	+
Steroids	+	+	-
Glycosides	-	-	-
Terpenoids	-	-	-
Flavonoids	+	+	+

+ = Present, - = Absent

FTIR Characterization

The FTIR spectrums of the CF, EF, and AF of ZSRB are presented in Figure 1, showing the various peaks. Exactly eight peaks were detected in the CF including three at the group frequency regions. The first absorption peak was at 3210.16 cm-1, corresponding to the O-H stretching frequency of the alcohol compounds while the second (1694 cm-1) and third (1605.65 cm-1) peaks at the frequency regions correspond to the C=C frequency of conjugated alkenes. In the fingerprint region, the peak at 1515.01 cm-1 (C-N stretch) corresponds to the amine groups. For the EF, eight peaks were detected including two at the group frequency region. The first absorption peak was at 3196.32 cm-1, corresponding to the O-H stretching frequency of the alcohol compounds. The second (1602.14 cm-1) peak at the frequency regions corresponds to the C=C frequency of conjugated alkenes. In the fingerprint region, the peaks at 1516.54 (N-O bending), 1436.80 (C-H bending), 1283.42 (C-N stretching), 1099.59 (C-N stretching), 1003.83 (S-O stretch), and 948.88 (C-H bend) fingerprint regions corresponds to the nitro compound, alkane, aromatic amine, amine, sulfonates, and monosubstituted alkene, respectively.

Exactly thirteen peaks were detected for the AF including three at the group frequency region. The first absorption peak was at 3226.50 cm-1, corresponding to the O-H stretching frequency of the carboxylic acid compounds. The second (2919.56 cm-1) and third (1605.72 cm-1) peaks at the frequency regions correspond to the C-H

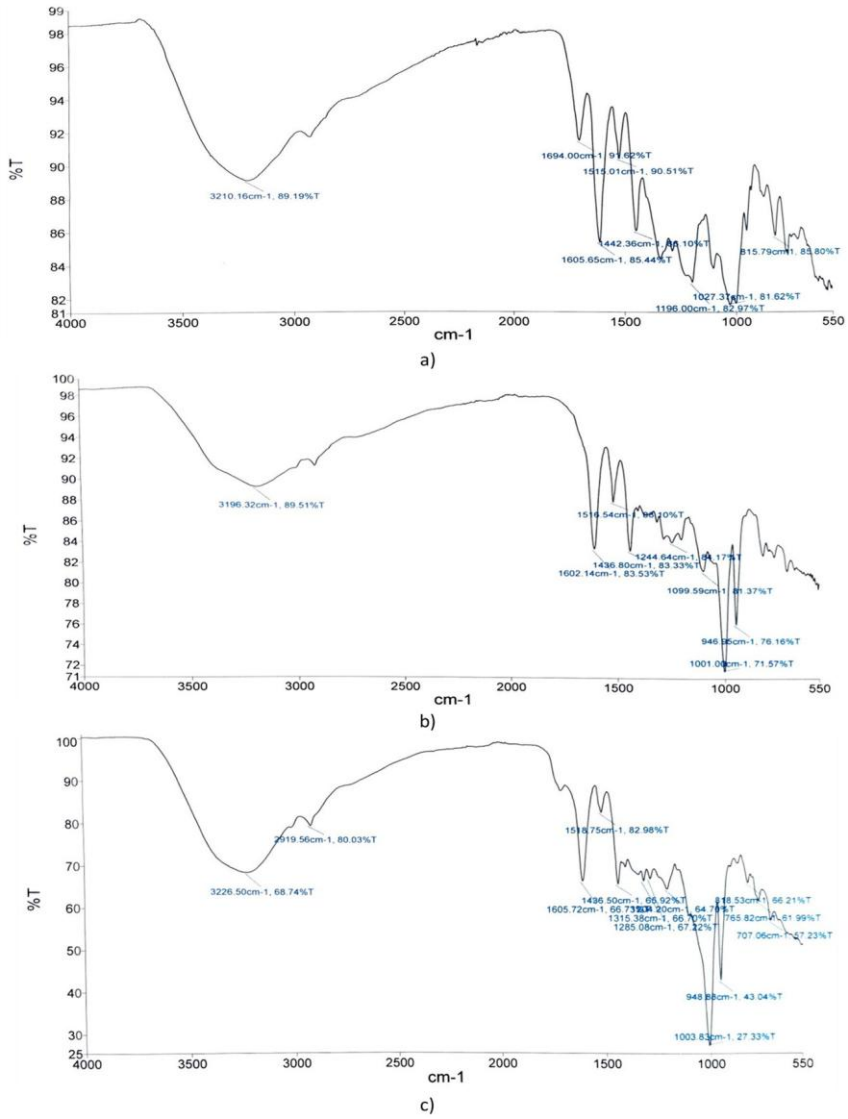

Figure 1. FTIR spectrum of ZSRB; a) CF, b) EF, and c) AF

stretching frequency of alkane and C=C frequency of conjugated alkene, respectively. In the fingerprint region, 1436.50 (C-N stretching), 1315.38 (O-H bending), 1285.08 (C-O stretching), 1003.83 (S-O stretching), 948.88 (C=C bending), 818.53 (C=C bending), 765.82 (C=C bending) and 707.06 cm-1 (C=C bending) correspond to the amines, phenol, aromatic ester, sulfoxide, *trans*-disubstituted alkene, trisubstituted alkene, disubstituted alkene, and *cis*- disubstituted alkene compounds, respectively.

Radical Scavenging Activity

Total Antioxidant Capacity (TAC)

Figure 2 presents the AA calibration curve and AAE TAC of the CF, EF, and AF of ZSRB. The EF and AF demonstrated a significantly (p < 0.05) higher TAC of 72.46 ± 0.55 µg/ml AAE and 71.51± 0.46 µg/ml AAE, respectively than CF (50.33 ±0.27 µg/ml AAE).

Total Reducing Power (TRP)

The AA calibration curve and AAE TRP of the CF, EF, and AF of ZSRB are shown in Figure 2. The AF exhibited a significantly (p < 0.05) higher TAC (54.07 ± 0.97 µg/ml AAE) than EF (42.76 ± 1.60 µg/ml AAE) and CF (30.13 ± 1.32 µg/ml AAE). Moreover, there was no significant (p > 0.05) difference between the CF and EF.

Ferric Thiocyanate (FTC)

Figure 2 shows the anti-lipid peroxidation activity of the CF, EF, and AF of ZSRB, depicting the percentage inhibition. A significantly (p < 0.05) higher percentage of inhibition was exhibited by

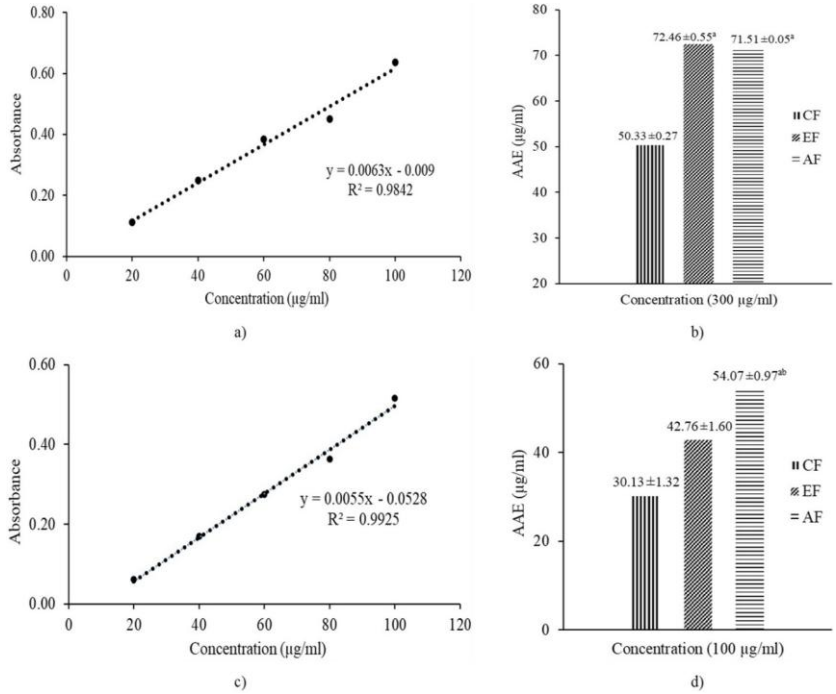

Figure 2. Radical scavenging activity of CF, EF, and AF of ZSRB; a) TAC AA calibration curve, b) AAE TAC, c) TRP AA calibration curve, and d) AAE TRP. Values with [a] and [b] superscripts are significantly higher than CF and EF, respectively.

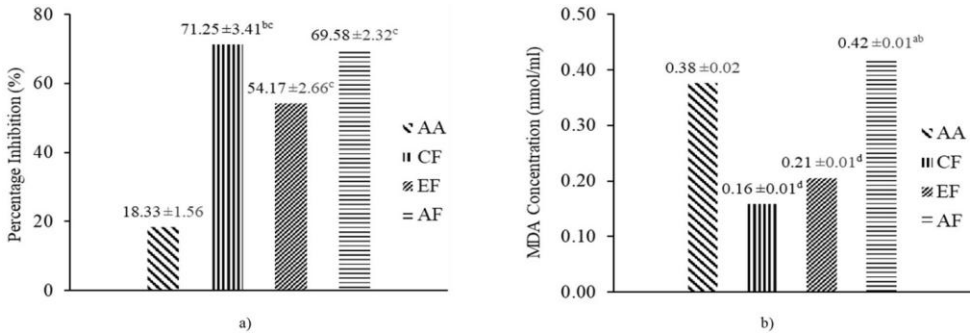

Figure 3. Anti-lipid peroxidation activity of the CF, EF, and AF of ZSRB; a) FTC method and b) TBA method. Values with [a] and [b] superscripts are significantly higher than CF and EF, respectively while Values with [c] and [d] superscripts are significantly higher and lower than AA, respectively.

the CF (71.25% ± 3.41) compared to the EF (54.17% ± 2.66). Moreover, all the fractions showed significantly ($p < 0.05$) higher activity than AA.

Thiobarbituric Acid Assay (TBA)

The anti-lipid peroxidation of the CF, EF, and AF of ZSRB *via* the TBA method is shown in Figure 2, revealing the MDA concentration. The CF (0.16 ± 0.01 nmol/ml) and EF (0.21 ± 0.01 nmol/ml) demonstrated significantly ($p < 0.05$) lower MDA concentration than the AF (0.42 ± 0.01 nmol/ml) and AA (0.38 ± 0.02 nmol/ml). Moreover, there was no significant ($p > 0.05$) difference between the MDA concentrations of CF and EF.

DISCUSSION

In the present study, the different fractions of ZSRB were subjected to secondary metabolite screening, and the antioxidant capability of each fraction was evaluated. The fractionation technique allows the separation of different secondary metabolites *via* partitioning based on solvent polarity and affinity for the solvents (Chóez-Guaranda et al., 2022). In our report, steroids were absent in the AF which might be due to the high affinity of the steroids towards the CH

and EF which were less polar than the AF. Thus, these compounds were extracted by the CF and EF, leaving none in the AF. The pharmacological activities of plant-based drugs can be attributed to the interaction of their secondary metabolites with targets linked to the pathology of many ailments.

The presence and nature of the secondary metabolites influence the associated pharmacological activity including free radical scavenging potential of the plants. The secondary metabolites reported in our study were previously linked to different antioxidant activities (Gul et al., 2017; Macáková et al., 2019; Cui et al., 2020; Muema et al., 2022; Rosero et al., 2022). These secondary metabolites were previously reported to mitigate oxidative stress in cancer by modulating redox balance and promoting cellular proliferation and survival (Gaikwad & Srivastava, 2022). In another study, they were reported to inhibit inflammatory molecules, increase antioxidant enzymes, and modulate signaling pathways (Culhuac et al., 2023). Thus, the antioxidant activities of the ZSRB might be attributed to these secondary metabolites.

The FTIR characterization further documents the presence of the different secondary metabolites *via* their functional groups. These functional groups are vital for the pharmacological activities of the plant including radical scavenging properties (Afieroho et al., 2020; Prakash et al., 2020; Quang et al., 2022; Gomathi & Maneemegalai, 2023). In our study, hydroxyl groups, alkenes, alkanes, nitro compounds, amine, and halo compounds were reported. Hydroxyl groups predominantly found in flavonoids demonstrate significant antioxidant properties *via* the single electron transfer or hydrogen atom transfer mechanism (Charlton et al., 2023). The hydroxyl groups form intramolecular hydrogen bonds to intercept peroxyl radicals, mitigating the oxidation effects of the radical (Rusina et al., 2022). Moreover, the positioning of the OH groups in the ortho and para positions of phenolic acids elevated their antioxidant activity (Rusina et al., 2022).

Aromatic amine groups are also important antioxidants exhibiting the single electron transfer mechanism and promoting the antioxidant activities of compounds *via* donating hydrogen atoms from the aromatic amino groups (Charlton et al., 2023). Sulfonate groups derived from chitosan were also reported to exert radical scavenging activity *via* hydroxyl-radical and superoxide-radical scavenging (Luan et al., 2018). Furthermore, Sulfonate groups in 6-amino-6-deoxychitosan exhibited a concentration-dependent hydroxyl radical scavenging activity (Luan et al., 2018). Additionally, the thio-sulfonates groups exhibit superior antioxidant activity than the sulfonates (Zenkov et al., 2007). The functional groups reported in our study might contribute significantly to the antioxidant effects of ZSRB fractions with each demonstrating either strength or weakness, depending on the test assay (Gomathi & Maneemegalai, 2023).

In our study, we reported the radical scavenging activity of different fractions of ZSRB in different assays *via* different methods. The EF and AF exhibited superior TAC than the CF which might be attributed to the superior polarity of the EF and AF solvents which extracts OH functional groups with superior hydrogen atom transfer and effective chain-breaking antioxidant capacity (Ballus et al., 2015). Moreover, this might also contribute to superior TAC exhibited by AA compared to the fractions. The TRP defines the capability to donate electrons and reduce other molecules (Zhang et al., 2015). Thus, in our report, the potential of the different fractions of ZSRB to reduce ferric ions was tested to ascertain the TAC of the fractions. The result showed a polarity-dependent increase in the reducing power of the fractions with the AF exhibiting the highest while the lowest exhibited by the CF. Moreover, AA exhibited superior reducing power than all the ZSRB fractions. The polarity of the solvent correlates with the efficiency of the antioxidant-reducing power of solvents, in addition to the medium involved (Sørensen et al., 2011; Zhong & Shahidi, 2012; Wakeel et al., 2019). In our study, the polar medium for the TRP might contribute to the polarity-dependent TRP of the fractions. Moreover, a reverse case was partly observed for the ant-lipid peroxidation assay in a non-polar medium.

The CF exhibited superior radical scavenging capability in both the FTC and TBA methods, demonstrating superior radical inhibition and lower MDA concentration than all the fractions and AA. The MDA concentration is a measure of lipid peroxidation in tissues, especially in the early stage of the process, and acts as an oxidative stress biomarker that indicates damage to macromolecules (Akbari et al., 2023). Thus, in our report, the CF might present a potential therapeutic against lipid peroxidation. Some of the anti-lipid peroxidation mechanisms of action of plants include free radical scavenging, monooxygenase inactivation, singlet oxygen quenching, and peroxidative metal chelating *via* antagonistic and synergistic mechanisms (Choe & Min, 2009; Waśkiewicz et al., 2014; He et al., 2015).

CONCLUSION

In our report, different fractions of ZSRB ranging from the non-polar to polar fractions were phytochemically characterized *via* FTIR and further evaluated for potential application in oxidative stress therapy. The characterization showed the presence of functional groups linked to radical scavenging activities. Moreover, the AF showed superior TAC and TRP while CF exhibited superior anti-lipid peroxidation potential. Conclusively, the ZSRB has potential antioxidant application in oxidative stress therapy with a focus on anti-lipid peroxidation for the CF though the AF has better TAC and TRP.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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