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Proximat composition, elemental analysis, phytochemical profile and evaluation of anti-bacterial and anti-fungal activity of stem bark of Buckthorn (*Rhamnus pentapomica* R. Parker)

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For the reason that antibiotic resistance is on the rise, people rely on conventional medicine for their basic medical requirements. A variety of bioactive chemicals found in medicinal plants can be used to treat multidrug-resistant bacteria. The objective of the study was to test medicinal plant phytochemical and biological assessment collected from District Orakzai named Sarha Wan [*Rhamnus pentapomica* R. Parker (Qaiser and Nazimuddin 1977)] by determining the proximate nutrient composition, elemental analysis, phytochemical and antimicrobial activity of its stem bark extract and fractions. The antibacterial and antifungal potentials of three dosages were determined using the Well Diffusion technique. The findings revealed that *R. pentapomica* retained moisture, ash, and nutrients in varying percentages, with a total nutritional value of 151.60 kcal/100 g. which would make it a potential nutraceutical. The examination of its powder sample indicated that it contains considerable amount of calcium, magnesium, iron, potassium, sodium, cobalt, zinc, lead, manganese and cupper. Results illustrates that methanol extract, chloroform and ethyl acetate fractions contained metabolites like carbohydrates, protein, tannins, alkaloids, flavonoids but in butanol fraction glycosides, fatty acid and protein were not detected. The Highest tannin level was found in methanol extract (27.5%) followed by alkaloid (7.5%), sterol (5.5%), flavonoid (3.25%) and saponins (2.5%). The methanol extract, chloroform and ethyl acetate fractions also showed better antibacterial effect at almost all doses than butanol fraction, with MIC values ranging from 125 to 1000 ug/ml. In antifungal activity, it was the chloroform fraction which showed dose dependent result against all the fungal strains at all concentrations. These findings suggest the use of active chemicals extracted from the stem bark of *Rhamnus pentapomica*, particularly the methanol extract, chloroform, and ethyal acetate fractions, as novel alternatives to the usage of certain drugs and as a substitute supply of antibiotics in pharmaceutical companies.

Keywords: Proximate Composition. Elemental analysis. Phytochemical profile. Antibacterial. Antifungal activities

INTRODUCTION

Rhamnus fairly is a large genus of 160 species, cosmopolitan in distribution. It is represented in Pakistan by 6 species only, distributed in the area of Baluchistan (Babar Mountain Zhob) and surroundings (Qaiser and Nazimuddin1977). *Rhamnus pentapomica* [*R. pentapomica* (Jehangir et al.2018)] known as “Buckthorn in English, Sarha Wan in Pashto” native to Pakistan, Afghanistan. India, Kashmir. This medicinal plant belongs to the Rhamnaceae family (Qaiser and Nazimuddin, 1977). The term ‘ *Rhamnus*’ comes from the Ancient Greek *rhámnos* (ῥάμνος) for various prickly shrubs, a thorny bush or small trees, commonly called buckthorn (Gaffiot 1934; Jehangir et al. 2018). This an evergreen thorny shrub is also found in Rakhni, District Barkhan, Baluchistan and Khee Kada hills at District Orakzai and Gosar hill at District Hangu, Khyber Pakhtunkhwa, Pakistan. Various species of *Rhamnus* is a popular remedy for various biological activities including antimicrobial (Berhanu 2014), antifungal (Manojlovic et al.2005), antioxidant (Gholivand and Piryaei 2014), antiproliferative (Jafari et al.2014), laxative (Clementi and Misiti2010). The leaves of *Rhamnus alaternus* showed antioxidant and free radical-scavenging properties (Ammar et al.2009). Fruits of *Rhamnus cathartica* are used in Bulgarian folk medicine as an antiseptic for wounds (Ashfaq et al.2017). Anthraquinone derived from *Rhamnus* are used as a pharmacological laxative (Das and Gezici 2018). *Rhamnus fallax* is used to treat skin diseases (Ashfaq et al.2017). *Rhamnus prinoides* is used

as a laxative, diuretic, preventive for syphilis, depurative, cholagogue, blood purifier, in treating pneumonia, colic problem, Gonorrhoea, strong antimicrobial and against the inflammation of the tonsils in children (Amabye2015). *Rhamnus alaternus* is used for the treatment of diabetes, hepatic and dermatological problems, antioxidants, antimutagenic, diuretic, laxative, hypotensive and anti-genotoxic activities (Ammar et al.2007).

Natural medicines have been utilized to improve health in many medical organizations since time immemorial, including Ayurveda, Unani, and Siddha (Kebede et al.2021). About 70 -80 percent of the world population in developing countries use some form of these complementary and alternative medicine at some point for their primary health care's (Wubetu et al.2017). In India, it is reported that conventional healers use 2,500 plant species and 100 species of plants serve as regular sources of medicine. 50% of the Pakistani population is normally treated by some 50,000 practitioners of conventional medication (Waheed et al.2020). In the past, a large number of antimicrobial compounds were discovered from synthetic and natural products for the treatment and control of infectious agents. However, only a few of them were reachable to the needy world's market (Kebede et al.2021). Antibiotics have saved millions of lives and led to significant increases in life expectancy over the previous century. However, the development of multi-drug resistance bacteria is threatening the therapeutic effectiveness of many current antibiotics. Antibiotic misuse accounts for a significant portion of the community's antibiotic burden, and it contributes significantly to the growing prevalence of resistance among important human diseases. The problem of resistance necessitates a fresh effort to test numerous medicinal plants for antibacterial properties (Abdalla et al.2020). Another motivator for scientists to look for new antimicrobial compounds from a variety of sources, including medicinal plants, is the increasing loss of plant species (Dahiya and Purkayastha 2012). Medicinal plants and aromatic plants is a valuable source of medicinal compounds with active substances contain a number of chemical constituents such as phytosterols, triterpenes, diterpenes, polyphenols, tannins alkaloids, flavonoids, saponins, glycosides and used as an important source of indispensable drugs with broad-spectrum antimicrobial activity (Le et al.2021).

The main objective of the present study was to quantify proximate nutrient composition (Idris et al.2019; Khan and Khan 2020), mineral content (Hseu 2004) in powdered *Rhamnus pentapomica* stem bark and to extract various phytochemicals in different organic solvent (Ingle et al.2017) and also to determine antibacterial and antifungal activities (Balouiri et al.2016) of MERP, CFRP, EFRP and BFRP fractions of stem bark of *Rhamnus pentapomica*, It may also provide evidence for the species' use as a new industrial crop in the cosmetic, pharmaceutical, and food sectors.

MATERIALS AND METHODS

Chemicals and reagents

Methanol, n- Hexane, Chloroform, Ethyl acetate, n-Butanol were purchased from Sigma-Aldrich Chimie (*S.Q.F*, *France*), Benedict's reagent, Molisch reagent, Million's reagent, Dragendrof's reagent, NaOH, HCl, FeCl2, KOH, nitric acid and per chloric acid, glacial acetic acid, acetic anhydride, ether, ammonium hydroxide, purified water, diethyl ether, sodium chloride, lead acetate, sulphuric acid, petroleum and DMSO were acquired from Department of Botany, Islamia College Peshawar. Mueller Hinton agar, Potato Dextrose agar and Ciprofloxacin and clotrimazole were bought from Universal chemicals *(Peshawar, Pakistan).*

Plant material

The stem bark of *Rhamnus pentpomica* was collected in the month of april (spring) from the hills of Khee Kada, Orakzai (Pakistan), located between 33° -33' to 33° -54' north latitudes and 70° -36' to 71° -22' east longitudes. The plant was identified by a taxonomist at the Department of Botany, Islamia College Peshawar, fixed on herbarium sheet, provided Voucher specimen number Yas-ICP-322 and submitted in the herbarium. The sample was dried in shade at room temperature and all impurities from the sample were removed by washing and rinsing with distilled water for 2-3 times. After drying, sample was grinded with electric grinder to a coarse powder and passed through fine sieve # 30. Packed in air tight plastic container and kept it in room for further analysis.

Determination of Proximate Composition

Powder of stem bark of *Rhamnus pentapomica* was used for nutritional analysis. On the collected species for proximate analysis, moisture, crude fiber, crude fats, total ash, carbohydrates, and proteins were by AOAC (1995) methods; Idris et al. (2019); Khan and Khan (2020).

The amount of organic matter was determined by subtracting the percentage of ash from one hundred (% Organic Matter = 100 - % Ash) and the carbohydrate content was measured by subtracting the values obtained for fat and protein from organic matter (Idris et al. 2019, Khan and Khan 2020)

Total % Carbohydrates=

$$(100 - \%\ crude\ protein + \%\ fibers + \%\ Fat + \%Moisture + \%Ash)$$

The sample's caloric value was determined using the "Atwater factor," which involved multiplying the value of crude protein, lipid, and carbohydrate by 4, 9, 4, and adding the results (Onwuka2005). The following formula was used to calculate the caloric value in (Kcal) (Total gross energy).

Energy value (Kcal/100 g) = (2.62 x %protein) + (8.37 x %fat) + (4.2 x % carbohydrate).

Elemental Analysis

Using hot mineralisation with nitric and per chloric acids, the mineral content of powdered *R. pentapomica* stem bark was determined (Hseu 2004). 20 mL nitric and perchloric acid were added after the mixture had fully evaporated, leaving a white hazy dry residue. The determination of four macro-elements (Ca, K, Mg, Na) and six micro-elements (Fe, Mn, Pb, Zn, Cu, and Co) was carried out using an atomic absorption spectrophotometer (Hitachi Z-8100, Japan). The results obtained are expressed as milligram per kilogram of Dry Weight (mg kg−1 DW).

Phytochemical screening of *Rhamnus pentapomica* stem bark extracts

Crude extract preparation and fractionation.

The preparation of *R. pentapomica* stem bark extracts was assessed using maceration extraction. To perform the maceration extraction,1kg of dry powder sample of stem bark was mixed with 2-2.5 litres of methanol for 3 days at room temperature and extracted the crude extracts during this period. After that, a rotary evaporator was used to concentrate the crude extract, and the rudimentary extract was dried completely in a water bath (Ingle et al.2017). For fractionation, the crude extract was separated in separating funnel by successive extraction using increasing polarity solvents: hexane, chloroform (CFRP), ethyl acetate (EFRP), and n-butanol (BFRP) for further phytochemical profile and antibacterial, antifungal test. The fractions were concentrated in a rotary evaporator. All of the fractions were vacuum-sealed under minimal pressure and held in clean glass bottles at room temperature (Ingle et al. 2017).

Testfor carbohydrates

For the detection of carbohydrates, Benedict's test was employed. After heating the extract solution with a few drops of Benedict's reagent on a water bath, a reddish brown precipitate forms, indicating that the sugar has been reduced (Evans2002). This was also confirmed by Molisch reagent test (Hassan et al.2020).

Test for proteins.

To determine the protein content, Million's test was employed. Colorations and precipitation were seen after treating solvent extracted materials with 2 ml of Million's reagent. The presence or absence of proteins or amino acids was determined whether or not a white precipitate became red following moderate heating (Evans 2002). For further conformation Ninhydrin Test was performed (Kumar and Kiladi 2009).

Test for tannins.

A small quantity of each extract was mixed with water, heated on water bath and filtered. A few drops of ferric chloride solution were added to the filtrate. A dark green coloration indicated the presence of tannins (Waheed et al.2020).

Test for alkaloids.

About 0.2 g of each extract was warmed with 2% H SO for 24 minutes. It was filtered and a few drops of Dragendrof's reagent were added. Orange red precipitate indicated the presence of alkaloids (Waheed et al.2020).

Test for flavonoids.

0.2 g extract was dissolved in diluted 10% NaOH and 2ml HCl was added. A yellow solution that turns colorless indicated the presence of flavonoids (Waheed et al.2020).

Test for cardiac glycosides.

Killaer Kilani test was applied in this case. Three to four millilitres of glacial acetic acid is added to an extract. One drop of FeCl3 (5%) and condensed H2SO4 solutions are added. The presence of steroidal glycosides can be shown by the appearance of a reddish brown color at the intersection of two liquid layers and a bright green color in the upper layer (Kebede et al.2021).

Test for phenoles.

Ferric chloride test was used**.** Apply a few drops of ferric chloride solution to the extract and dissolve it in 2 mL ethanol. Phenols manifest themselves as a bluish black or deep bluish green color (Kebede et al.2021).

Test for saponine.

Frothing test was used. In a test tube, combine 2ml of plant extract with 2-3ml of water and vigorously shake. The existence of saponine is shown by the formation of persistence froth (Kebede et al. 2021).

Test for phytosterol and triterpenoids.

Following Libermann-Burchard test2 mL of acetic anhydride was added to 0.5 g of each extract and then added 2 mL of H2S04. The color changed from violet to blue or green or red which indicated the presence of steroids and the presence of triterpenoids can be determined by the formation of a deep red color (Waheed et al.2020).

Test for Fatty acid.

Prepare a 0.5 mL extract solution by treating it with 5 mL ether, then pouring it onto filter paper and allowing it to dry completely. The presence of fatty acids is shown by the forming of transparency on filter film (Ayoola et al.2008).

Total alkaloids quantification.

Total percent alkaloids were determined by following the protocol of Harborne (1998). In a beaker, 2 gram of methanolic extract was dissolved in 100 ml of 10% acetic acid and held for 4 hours after coating. The mixture was condensed in a water bath until it reached 1/4th of its original amount, after which concentrated ammonium hydroxide was applied drop by drop for precipitation. This precipitate was deposited on Whitman filter paper that had been pre-weighted (W1) and then washed with dilute

ammonium hydroxide. The residual, as well as the filter paper, was dried, weighed (W2), and the volume of alkaloid in mg/g and percent was measured.

Total saponin quantification.

For total saponin quantification Obadoni and Ochuko (2002) model was adopted.20 ml purified water was used to dilute 2 gram of methanolic extract. The mixture was then transferred to a 250 mL separation funnel, which was then filled with 20 mL diethyl ether and vigorously shaken. The aqueous layer was diluted with 60 ml n-butanol after the ether layer was removed, and saponin precipitated. The saponin was collected on Whitman filter paper that had been pre-weighted (W1) and washed twice with a 5 percent aqueous sodium chloride solution. The precipetate was then dried to a constant weight (W2) in an oven (40°C), and the saponin content in mg/g as a percentage of the original weight of the sample taken was measured.

Total tannins quantification.

Following the method of Carmona et al. (1991), total tannins were quantified. To make a suspension, 2 gram of methanolic extracts is liquefied in 75 ml distilled water. The suspension was then prepared with a saturated solution of lead acetate to produce tannins as a lead-tinnate precipitate. The residue (lead-tinnate precipitate) was then dissolved in 20 mL purified water, and the lead sulphate was removed on acidification by applying an excess of dilute sulphuric acid. Finally, the tannins were extracted on pre-weighted Whitman filter paper (W1), dried in a hot air oven at 60oC, and the quantity of tannin was measured by taking the filter paper's weight once more. Tannins content in mg/g as well as percentage of the initial weight of sample taken was calculated.

Total sterols quantification.

Following the method of Kokate (1994), total sterols were quantified.75ml purified water was used to dilute 2 gram of methanolic extract. Chlorophylls are converted to water soluble salts or chlorophyllins by adding 25 mL of 10% KOH to the mixture. In a separatory funnel, this mixture was removed three times with 75ml petroleum ether each time. The ether layer was moved from each extraction into a pre-weighted beaker (W1) and held on a water bath to fully evaporate the solvent. The sterol content remained in the bottom of the flask, so it was weighted along with the contents (W2) and the volume in mg/g and percentage were calculated (Huang et al. 2010).

Total flavonoids quantification.

Following the method of Mir et al. (2013), total flavonoids were quantified. Approximately 5g of plant extracts is dissolved in 100ml of 80% aqueous methanolic extracts and kept in the refrigerator overnight. In the next day, drop by drop, chloroform (for glycosides flavonoids) or ethyl acetate (for aglycosides flavonoids) was applied to the solution, and the mixture was transferred to the pre-weighted beaker (W1). It was put on a water bath to evaporate the solvents and then weighted to finish drying (W2). The amount and percentage is determined.

Amount and % of Phytochemicals (alkaloids, saponin, tannins, sterole and flavonoids) were determined by using the formula below.

$$\text{Amount of Phytochemicals (mg/g)} = \frac{X}{\text{Weight of sample}}$$

$$\%\text{ of Phytochemicals} = \frac{X}{\text{Weight of sample}} \times 100$$

Here, X = Weight of Phytochemicals = W2- W1

W1 = Weight of filter paper/ flask/beaker

W2 = Weight of filter paper/flask/beaker + residue

Determination of anti-bacterial activity of *R. pentapomica* stem bark extracts

Experimental pathogens used.

Seven different microbial strains, causing infective and toxic food poisoning were obtained from the "Medicinal Botanical Centre (MBC)" PCSIR Lab. Peshawar and Agriculture University, Peshawar, Pakistan. For Gram +ve bacterial strains *Bacillus atrophaeus*, *Bacillus subtilis* and *Staphylococcus aureus* and for Gram –ve bacterial strains *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, and *Klebsiella pneumoniae* were selected.

Inhibitory zone assay (agar well diffusion method).

To perform the agar well diffusion method, 20 mL of Mueller Hinton agar was tipped into disinfected petri plates. The agar media was permitted to get hard in platters and inoculated with 200µL of bacterial suspension. Then a hole with a diameter of 6 to 8mm at an equal distance (25mm) among two separate wells is punched aseptically with a sterile cork borer or a tip, and a volume, 1mg 6 ul−1 well−1, 2 mg 12 ul−1 well−1 and 3 mg 18 ul−1 well−1 of plant solvent extracts of each fraction were added into the wells and allowed to diffuse at room temperature for 2 hrs and then the plates were incubated at 37°C for 18-24 h for bacterial pathogens. The Ciprofloxacin (30 µg ul−1) was used as a positive control, while the DMSO was used as a negative control (Balouiri et al.2016). Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were recorded. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested. The diameter of the zone inhibition measured in millimetre (mm) correlates to the sensitivity of the strainto the extract and was calculated by measuring the clear area (where, the bacterial growth was not occurred) around the wells. The percent inhibition was recorded using following formula.

$$\text{Zone of Inhibition (\%)} = 100 - \frac{MC - MT}{MC} \times 100$$

Where, MC= Mean zone of inhibition by standard drug Ciprofloxacin

MT = Mean zone of inhibition by test drugs

Determination of minimum inhibitory concentration (MIC).

The minimum inhibitory concentration (MIC) of the extracts (MERP, CFRP, EFRP and BFRP in 5%DMSO) was determined using the broth dilution method reported by Manandhar et al. (2019) with slight modifications. Twofold serial dilutions of the different Extracts of stem bark were prepared (1000, 500, 250, 125 and 62.5 ug ul−1), then 0.2 mL of the different bacterial suspensions (0.5 McFarland broth inoculum) were added to each test tube except the negative control (CTR-) and tubes were incubated for 24 h at 37 °C.The Ciprofloxacin (62.5 µg ul−1) was used as a positive control, while the DMSO was used as a negative control. MIC values were determined by turbidity test. The lowest concentration of the extract inhibiting the growth of microbial strains with clear suspension was considered as the MIC values. The test was performed in triplicates.

Determination of anti-fungal activity of *R. pentapomica* stem bark extracts

Fungal Strains used. One strain of human and six strains of plant pathogenic fungi, *Candida albicans, Pythium debrayanum, Aspergillus niger, Aspergillus flavus, Alternaria alternnata, Alternaria solani,* and *Fuserium oxsysporium* were used for the experiment provided by "Medicinal Botanical Centre (MBC)" PCSIR Lab. Peshawar and Agriculture University, Peshawar, Pakistan.

Agar well diffusion method.

To perform the agar well diffusion method for antifungal activity, protocol of (Olurinola 1996) with slight modification was used. 20- 25 ml of potato dextrose agar (PDA) was poured into disinfected petri plates. The PDA was permitted to get hard in platters and inoculated with 200 µL of fungal suspension. Then a hole with a diameter of 6 to 8mm at an equal distance (25mm) among two separate wells is punched aseptically with a sterile cork borer or a tip, and a volume, 6ul, 12ul and 18ul ml (1 mg/6ul, 2mg/12ul and 3mg/18ul) of plant solvent extracts of each fraction (MERP, CFRP, EFRP and BFRP) were added into the wells and allowed to diffuse at room temperature for 2 hrs and then the plates were incubated at 28°C for three days. The Clotrimazole (30 µg mL− 1) was used as a positive control, while the DMSO was used as a negative control. The experiment was performed in triplicates and the inhibition of mycelia growth was observed on 3rd day. Data was recorded as diameter in mm and was documented as Mean and SEM. Fungal mycelium reduction by the tested drugs was compared with the standard antibiotic control Clotrimazole (Umadevi et al. 2018). The percent inhibition was recorded using following formula.

Mycelium growth Inhibition (%) =100- $\frac{\mathrm{MzC}-\mathrm{MzT}}{\mathrm{MzC}} \times 100$

Where, MzC= Mean zone of inhibition of standard drug Clotrimazole

MzT = Mean zone of inhibition of test drugs.

Statistical analysis

Extracts were analyzed in triplicate (n = 3) for all of the parameters examined below. The one-way analysis of variance (ANOVA) in SPSS software was utilized for statistical analysis, with the Dunnet test applied at a significance level of $p < 0.05$. Then data has been converted into perntage.

RESULTS

Proximate composition

proximate composition was determined in powdered *R. pentapomica* stem bark and the results obtained are presented in Table 1. The results achieved indicated that powdered *R. pentapomica* stem bark contain least % ratio of fat content (0.65% DW) which is similar to the finding of Ajayi et al. (2018) who revealed fat contents in *Brassica oleracea* and *Solanum macrocarpon*. The highest protein content was found is (8.86% DW) which are above the critical value of 7.0% as reported by Kafeel et al. (2013). A protein content of the recent study is higher to the finding of Adnan et al. (2010). The highest concentarion of carbohydrates was found (46.93% DW) are in line with the carbohydrate content in the leaves of *Ficus capensis* previously investigated by Achi et al. (2017). The maximum crude fibres (CF) and neutral detergent fibre (NDF) content was recorded in stem bark (28.92% DW and 55.17 % DW) respectively. Powdered of *R. pentapomica*stem bark is adequate in ash contents (7.34% DW) and this proudly signifies that this plant isgifted with good amount of minerals Aborisade et al. (2017). The moisture contents set up for stem bark is (7.3%) which is very important because plant which falls below the 15% moisture contents are said to be a safe storage limit for food materials. Thus plants with lower moisture contents can be stored for long without fear of deterioration Osunlana et al. (2018). Plant crude food products that provide more than 10% energy caloric value are advised to be rich source of proteins. Nutritional value as well as energy of the medicinal plants is generally used to exploit medicinal plant as good component of food (Khan *et al.,* 2020). The mean gross energy of the stem bark was highest (151.60 Kcal/100g). Our findings are confirmed by the work of Khan and Khan (2020). The proximate compositions of stem bark of *Rhamnus pentapomica* value for the mean dry matter, moisture content, ash, crude protein, crude fibre, crude fats and carbohydrates for summer season investigated in the recent study are also comparable with the values reported

by Amabye (2015).

Table 1: Proximate composition of powdered stem bark of *Rhamnus pentapomica*

Composition (%)	*Stem Bark of R. pentapomica*
Moisture	7.3
Ash	7.34
Crude Fat	0.65
Crude Protein	8.86
Carbohydrates	46.93
Crude Fibres	28.92
NDF	55.17
Gross Energy (Kcal/100g) Mean ± SEM	151.60

Results moisture, ash, crude fat, crude protein, carbohydrates, crude fibres and NDF are expressed in dry weight (DW) except for moisture and all refer to the average of three determinations ± SD
NDF neutral detergent fibre

Elemental composition

Elemental composition was determined in powdered *R. pentapomica* stem bark and the results obtained are presented in Table 2. The results obtained showed that powdered *R. pentapomica* stem bark mount up important minerals contents. Considerable amount of Calcium (4269 ± 0.221mg/kg) was recorded for stem bark, followed by Magnesium (358.6 ± 0.7511mg/kg), Iron (139.65 ± 0.0484mg/kg), Potassium (1048 ± 0.362mg/kg), Sodium (117.6 ± 0.0031mg/kg), Cobalt (10.9 ±0.0762mg/kg), Zinc (9.15 ± 0.0066mg/kg), Lead (9 ± 0.0308mg/kg), Manganese (8.75 ± 0.0.0062mg/kg) and Cupper (2.55 ± 0.0082mg/kg). There are numerous papers in the literature on mineral amount analyses in various species, but no information about the mineral composition of *R. pentapomica* stem bark, which encouraged us to conduct this research. For instance, Abdoul-Azize (2016) determined the mineral composition of *Ziziphus lotus* from Rhmnaceae family and reported that K, Ca, Na, Fe, and Zn contents in *Z. lotus* seed were lower than contents in stem bark of *R. pentapomica* with value estimated at 1048, 4269, 117.6, 139.65, and 9.15 mg kg−1 DW, respectively while Mg, and Mn contents were higher than contents in stem bark of *R. pentapomica* with value estimated at 358.6 and 8.75 mg kg−1 DW, respectively. Yerima and Adamu (2011) studied the mineral composition of *Ziziphus mauritiana*, a Rhmnaceae species, and found that the K and Na contents in *Z. mauritiana* were greater than those in *R. pentapomica* stem bark, with values of 1048 and 117.6 6 mg kg−1 DW, respectively.

The minimum daily intake of K and Na are 3.5 and 2.4 g and below the required intake may lead to body weight loss and nerve disorder (Achi et al.2017). Minerals found to be present in trace quantities are Co, Zn, Pb, Mn and Cu. Their high amounts are not ideal and not desirable for the functioning of the body (Achi et al.2017).

Table 2: Concentrations of inorganics macro- and microelements (mg.kg−1 DW) in powdered *R. pentapomica* stem bark

Class	Sr#.	Elements	Stem bark of *R. pentapomica*	
			Absorbance (Wavelength)	Weight mg/kg)
Macro -nutrients	1	Calcium	422.7	4269 ± 0.22
	2	Potassium	766.5	1048 ± 0.36
	3	Magnesium	285.2	358.6 ± 0.75
	4	Sodium	589	117.6 ± 0.003
Micro -nutrients	5	Iron	248.3	139.65 ± 0.048
	6	Manganese	279.5	8.75 ± 0.0.006
	7	Lead	283.3	9 ± 0.030
	8	Zinc	213.9	9.15 ± 0.006
	9	Cupper	324.8	2.55 ± 0.008
	10	Cobalt	240.7	10.9 ± 0.076

Results are expressedas the average of three determinations ± SEM.

Cobalt (Co) being a trace element it is very necessary in all mammals for the normal functions of the body and in human it is used to treat several different types of cancer and anaemia but the intake of high amount can cause heart diseases (Haq et al.2012). Moderate % of Zinc is vital in transcription, translation, cellular differentiation and immunity and tonic (Achi et al.2017). This justifies the use of *Rhamnus pentapomica* in folklore medicine because of the presence of desirable amount of elements and their beneficiary effects.

Major Phytochemicals

The different phytoconstituents determined in stem bark of *R. pentapomica* are represented in Table 3and Table 4. The phytochemical screening of various extracted samples MERP, CFRP, EFRP and BFRP of stem bark of *Rhamnus pentapomica* in the present study showed that this plant is a rich source of alkaloids, phenol, flavonoids, saponine, tannins and steroids. Glycosides, fatty acid and protein were detected in almost all fractions except BFRP fraction. The presence of key bioactive components in various quantities is ensured by quantifying phytochemicals such as alkaloids, flavonoids, sterole, saponine, and tannins in crude methanolic extract of stem bark of *Rhamnus pentapomica*. According to Khan and MA (1972) endeavor to develop *Rhamnus pentapomica* as a new source of tannin, the maximum tannin concentration investigated in the MERP extract of stem bark was assessed to be 275 ± 0.61 mg/g. The maximum alkaloids contents found in the MERP extract was valued at 75 ± 0.34mg/g, followed by flavonoids 65 ± 0.43 mg/g, sterole 55 ± 0.51mg/g, and saponine 15 ± 0.38 mg/g DW. Different Rhmnaceae species were studied for phytochemicals, but it was the first time that *R. pentapomica* stem bark was studied qualitatively and quantitatively. Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include

tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids which further synthesis complex chemical substances Hasan et al. (2019). These photochemical have vast range of biodiversity in same as well as different species and are stored in various plant parts like stem, root, leave, flowers, bark etc. that are not utilized by the plants, but are very essential for humans due to their therapeutic properties Das and Gezici (2018).

Antibacterial activities

The bacterial strains evaluated for sensitivity to *R. pentapomica* MERP extract, CFRP, EFRP and BFRP fractions of stem bark was determined after 24 h of incubation at 37°C. The area of zone of inhibition developed around the well treated with extracts was measured and the result obtained is presented in Table 5.

According to the results obtained, MERP extract, CFRP, EFRP and BFRP fractions of stem bark of *Rhamnous pentapomica* (1, 2 and 3mgul−1well−1) showed good inhibitory activity against *Staphylococcus aureus*. The CFRP fraction showed better antibacterial activity than the MERP, EFRP and BFRP fractions. The best antibacterial activity of the CFRP fraction was against *S. aureus* strain with % inhibition zone estimated at 63.29%, 64.6% and 67.4% at concentration of 1, 2 and 3mg ul−1well−1 followed by EFRP fraction 54.7%, 64.2% and 67.4% at dose dependant manner respectively. MERP extract, CFRP, EFRP and BFRP fractions were also showed better results against *Bacilus subtilis*. The highest % zone of inhibition was shown by MERP extract 55.33%, 66.6%, 74.33%followed by CFRP fraction61%, 66.6%, 66.6% at concentrations of 1, 2 and 3mg ul−1well−1 respectively. MERP, CFRP and EFRP fractions were found effective against *Bacillus atropheus* at all tested doses. The highest % zone of inhibition was recorded for EFRP fraction 85% followed by CFRP fraction 80% and MERP extract 71.5%at concentration of 3mg ul−1well−1. MERP, CFRP and EFRP fractions also displayed major results against *Pseudomonas aeruginosa* at all concentrations. The highest % zone of inhibition was recorded by MERP extract 62.5%, 65.65, 71.87% at concentration of 1, 2 and 3mg ul−1well−1 respectively followed by CFRP and EFRP fractions with higest value 62.5% and 56.25% at 3mg ul−1well−1 respectively. MERP extract and EFRP fraction were thoroughly effective against *Escherichia coli*58.6% and 51% respectively at concentration of 3mg ul−1well−1 while BFRP fraction inhibited the growth of *Escherichia coli* by 65.3% at concentration of 3mg ul−1well−1. However, CFRP fraction did not show any antibacterial activity against *Escherichia coli.* The growth of *Salmonella typhi* was also fairly restricted by MERP extract, CFRP and EFRP fractions showing good antibacterial activity at all concentrations. The highest %zone of inhibition was recorded for CFRP fraction 76.07% at concentration 3mg ul−1well−1 followed by MERP extract71.8% and EFRP fraction 64.28%at 3mg ul−1well−1 respectively. MERP extract, CFRP and EFRP fractions were also found effective against *Klebsillea pneumonia* at all concentrations. On the other hand, BFRP fraction did not display any inhibitions against *Klebsillea pneumonia* at all three concentrations. The highest % zone of inhibition was noted by CFRP fraction56% at concentration of 3mg ul−1well−1. The detected antibacterial activity of the fractions are highly analogous with the Ciprofloxacin (30µg ul−1well−1), considering 100% inhibition, against *S. aureus* with the zone of inhibition (31.66±.88mm) followed by *B. subtilis* (30.00±.57mm), *B. atrophaeus*, (20.00±.57mm), *P. aeruginosa* (32.00±.57mm), *E. coli* (30.00±.57mm), *S. typhi* (28.00±.57mm) and *K. pneumonia* (30.00±1.15mm).

Minimum inhibitory Concentration.

The minimum inhibitory concentration (MIC) assay was assessed for only the bacterial strains that showed sensitivity to *R. pentapomica stem bark extracts* (1mg ul−1 well−1) in the well diffusion method previously performed and the results obtained are in Table 6.

Table 6 shows the turbidity of the penicillin, *R. pentapomica* Stem bark inoculations, and positive (CTR +) and negative (CTR −) controls after 24 h of incubation. The CTR +containing the broth nutrient, bacterial culture and antibioticor *R. pentapomica* extracts showed turbidity (bacterial growth) after the 24 h of incubation and was used to test the growing ability of the medium, while the CTR − containing only the broth nutrient and the antibiotic or *R. pentapomica* extract didnot show turbidity (no bacterial growth) after the 24 h of incubation and was used to test the sterility of the mediumand equipment.

The MERP showed minimum inhibitory concentration against *S. aureus* (250 ug ul−1), *B. subtilis* (500 ug ul−1), *B. atrophaeus* (500 ug ul−1), *P. aeruginosa* (250 ug ul−1), *E. coli* (125 ug ul−1), *S. typhi* (500 ug ul−1) and *K. pneumonia* (1000 ug ul−1). For CFRP and EFRP the MIC values recorded were *S. aureus* (250 ug ul−1 each), *B. subtilis* (500 ug ul−1and 1000 ug ul−1), *B. atrophaeus* (500 ug ul−1and 250 ug ul−1), *P. aeruginosa* (1000 ug ul−1each), *E. coli* (0 and 1000ug ul−1), *S. typhi* (1000 ug ul−1and 250 ug ul−1)and *K. pneumonia* (500 ug ul−1and 1000 ug ul−1)respectively as presented in table (7). The strong activities against the pathogenic strains are due to the presence of various secondary metabolites like phenol, alkaloids, saponine etc investigated during the phytochemical screening earlier. Data of the recent study showed that these various fractions MERP, CFRP, EFRP and BFRP are a good source of anti-pathogenic bacteria which may be used in flockier medicines for various ailments like pulmonary trouble (bronchitis), pyrexia, sinusitis, pneumonia and many more related diseases.

Table 3: Preliminary qualitative screening of *Rhamnous pentapom* MERP extract, CFRP, EFRP and BFRP stem bark fractions

Sr#	Phytochemicals	Chemical test	MFRP	CFRP	EFRP	BFRP
1	Carbohydrate	Fehling's test	+	+	+	+
		Benedict's test	+	+	+	+
2	Protein	Million's test	+	+	+	-
		Ninhydrine test	+	+	+	-
3	Tannin	Ferricchloridetest	+	+	+	+
		Gelatin test	+	+	+	+
		Alkali reagent	+	+	+	+
		Lead acetate test	+	+	+	+
4	Alkaloid	Wagner solution t	+	+	+	+
		Mayer's test	+	+	+	
		Dragendroff's	+	+	+	+
		Hager solution	+	+	+	+
5	Flavonoids	Alkali reagent	+	+	+	+
		Shinoda test	+	+	+	+
		Ammoniasolution test	+	+	+	+
6	Glycosides	Salkowski test	+	+	+	-
7	Phenole	Killaer-kilani test	+	+	+	+
		Ferric chloride	+	+	+	+
8	Saponins	Frothing test	+	+	+	+
9	Terpenoid	Chloroform test	+	+	+	+
10	Steroid	Sulfuric acid test	+	+	+	+
11	Phytosterols	Libermann-Burchard test	+	+	+	+
		Salkowski's test	+	+	+	+
12	Fatty acid		+	+	+	-

Table 4: Preliminary Quantitative analysis of methanolic extract of Stem bark of *Rhamnous pentapomica*

MERP	Flavonoids		Alkaloids		Sterol		Saponines		Tannins	
	Amount (mg/g)	%	Amount (mg/g)	%	Amount (mg/g)	%	Amount (mg/g)	%	Amount (mg/g)	%
Value	65 ± 0.435	3.25	75 ± 0.340	7.5	55 ± 0.517	5.5	15 ± 0.384	1.5	275 ± 0.616	27.5

Results represent the mean of three replicates (n = 3) and are expressed as mean value ±SEM.

Table 5: Mean percent inhibition of bacterial growth produced around the well treated with *R. pentapomica* MERP extract, CFRP, EFRP, and BFRP fractions (1mg, 2mg and 3mgul−1 well−1

Pathogens	Conc. mg/well	Mean % Inhibition of bacterial strains by*R.pentapomica*			
		MERP	CFRP	EFRP	BFRP
S.aureus	1mg	53.79	63.29	54.7	30.39
	2mg	54.74	64.6	64.2	31.64
	3mg	58.6	67.4	67.4	33.54
B.subtilis	1mg	55.33	61	50	NS
	2mg	66.6	66.6	53.3	37.6
	3mg	74.33	66.6	53.3	40
B.atropheus	1mg	60.2	75	70	NS
	2mg	61.5	76.5	83	NS
	3mg	71.5	80	85	NS
P.aeruginosa	1mg	62.5	46.87	40.62	NS
	2mg	65.6	51.87	40.87	31.25
	3mg	71.87	62.5	56.25	31.25
E.coli	1mg	50	NS	32	NS
	2mg	52	NS	48.6	60
	3mg	58.6	NS	51	65.3
S.typhi	1mg	71.4	71.4	52.1	NS
	2mg	71.6	71.4	53	NS
	3mg	71.8	76.07	64.28	NS
K.pneumonia	1mg	32	51	44	NS
	2mg	37.6	53.3	52	NS
	3mg	40	56	54	NS

Results are % means of three different experiments (*n* = 3). Ciprofloxacin (30 ug ul−1 well−1) was used as a positive control. DMSO was used as negative control. No inhibition zone is indicated by letter NS (Not Sensitive)

Antifungal activity

The sensitivity of fungal strains against *R. pentapomica* stem bark extract was determined after 48 h of incubation at 28 °C. The area developed around the well treated with extracts was measured and the results obtained are represented in Table 8. According to the results gained, the MERP extract, CFRP and EFRP fractions of stem bark of *Rhamnous pentapomica* (1, 2, and 3 mg uL−1 well−1) showed good inhibitory effect against the fungal growth. MERP extract, CFRP and EFRP fractions showed better antifungal action against *Candida albicans* at all concentrations at dose dependent manner. However, BFRP fraction did not show any antifungal activity against *C. albicans*. The maximum % inhibition zone recorded for CFRP fraction valued at 93.7% followed by EFRP fraction 89% and MERP extract 78.1%at dose 3 mg ul−1 well−1respectively. CFRP and BFRP fractions also inhibited the growth of *Pythium debaryanum* at all concentration and the highest %zone of inhibition was recorded for CFRP and BFRP fractionestimated at 54.1%and 46.45%at concentration of 3 mg ul−1 well−1 respectively. Though, MERP extract and EFRP fraction did not display any antifungal activity against *Pythium debaryanum.*

Best antifungal activity was recorded for MERP extract against *Aspergillus niger* with valued 70.9% at concentarion of 2 and 3 mg ul−1 well−1 followed by EFRP and CFRP fractions valued at 68.9% and 59.8% concentration of 3 mg uL−1 well−1 respectively. On the other hand, BFRP fraction did not exhibit any antifungal activity against *Aspergillus niger*at any dose concentration. The top antifungal activity of CFRP fraction was observed against *Aspergilus flavus, Alternaria alternate* and *Alternaria solani*with % inhibition zone estimated at 74.5%, 41.1% and 40.5% at concentration of 3 mg ul−1 well−1 respectively. Conversely, MERP extract, EFRP and BFRP fractions did not give away any antifungal activity against *Aspergillus flavus, Alterneria alternata* and *Alterneria solani* at all tested concentrations. Fungal strain, Fusarium *oxysporum,* was found susceptible to MERP extract, CFRP, EFRP and BFRP fractions and the maximum % inhibition zone observed for CFRP fraction estimated at 56%followed by EFRP 46.7%, MERP 35.9% and BFRP fractions 30.5% at concentrations of 2 and 3 mg ul−1 well−1 respectively. The resolute antifungal activity of methanolic extract, CFRP, EFRP and BFRPR fractions are highly analogous with standard antibiotic Clotrimazole (50ugul−1 well−1), considering 100% inhibition, against *Candida albicans* with the zone of inhibition (32.00±1.15mm), *Pythium debaryanum* (39.33±.66mm), *Aspergillus niger* (42.33±1.45mm), *Aspergillus flavus* (36.66±1.6mm), *Alternaria alternata* (47.66±1.45mm), *Alternaria solani* (49.33±0.66mm), and *Fuserium oxsysporium* (37±1.5mm).

Table 6: Turbidity of the Ciprofloxacin, *R. pentapomica* stem bark inoculations after 24 h of incubation at 37 °C

Cc (ug mL−1)	Ciprofloxacin					*R. pentapomica* MERP extract					*R. pentapomica* CFRP fraction					*R. pentapomica* EFRP fraction					*R. pentapomica* BFRP Fraction					CTR+	CTR-
	62	125	250	500	1000	62	125	250	500	1000	62	125	250	500	1000	62	125	250	500	1000	62	125	250	500	1000		
S.aureus	-	-	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	-	-	-	+	+	+	+	-	+	-
B.subtilis	-	-	-	-	-	+	+	+	-	-	+	+	-	-	-	+	+	+	+	-	NT					+	-
B.atropheus	-	-	-	-	-	+	+	+	-	-	+	+	+	-	-	+	+	-	-	-	NT					+	-
P.aeruginosa	-	-	-	-	-	+	+	-	-	-	+	+	+	+	-	+	+	+	+	-	NT					+	-
E.coli	-	-	-	-	-	+	-	-	-	-	NT					+	+	+	+	-	NT					+	-
S.typhi	-	-	-	-	-	+	+	+	-		+	+	+	+	-	+	+	-	-	-	NT					+	-
K.pneumonia	-	-	-	-	-	+	+	+	+	-	+	+	+	-	-	+	+	+	+	-	NT					+	-

(+) Turbidity indicating bacterial growth, (−) no turbidity indicating no bacterial growth and NT indicates microbial strain not tested

Table 7: MIC values of the Ciprofloxacin and *R. pentapomica* extracts against the different bacterial strains

Pathogens	MIC (ug ul−1)				
	Ciprofloxacin	MERP extract	CFRP fraction	EFRP fraction	BFRP fraction
S.aureus	62	250	250	250	1000
B.subtilis	62	500	250	1000	NT
B.atropheus	62	500	500	250	NT
P.aeruginosa	62	250	1000	1000	NT
E.coli	62	125	NT	1000	NT
S.typhi	62	500	1000	250	NT
K.pneumonia	62	1000	500	1000	NT

MIC of all extracted sample at all concentrations is lower than the lowest concentration of the antibiotic in the tube, NT indicates microbial strain not tested

Table 8: Mean percent inhibition of fungal growth produced around the well treated with *R. pentapomica* MERP extract, CFRP, EFRP, and BFRP fractions (1mg, 2mg and 3mgul−1well−1

Pathogens	Conc. (mg/well)	Mean % Inhibition of fungal growth by *R pentapomica* stem bark extracts			
		MERP	CFRP	EFRP	BFRP
C. albicans	1	69.6	92.5	79	NS
	2	76.8	93.7	80	NS
	3	78.1	93.7	89	NS
P. debaryanum	1	NS	36.3	NS	31.2
	2	NS	38.9	NS	41.4
	3	NS	54.1	NS	46.5
A. niger	1	67.6	30.7	59.1	NS
	2	70.9	54.3	61.4	NS
	3	70.9	59.8	68.9	NS
A. Flavus	1	NS	40.9	NS	NS
	2	NS	58.1	NS	NS
	3	NS	74.5	NS	NS
A. alternata	1	NS	24.3	NS	NS
	2	NS	34.8	NS	NS
	3	NS	41.1	NS	NS
A. solani	1	NS	18.2	NS	NS
	2	NS	27.5	NS	NS
	3	NS	40.5	NS	NS
F. oxysporum	1	29.7	51	37	25
	2	32.4	56	45.9	28.6
	3	35.9	56	46.7	30.5

Results are % means of three different experiments (*n* = 3). Clotrimazole (50 ug ul−1 well−1) was used as a positive control. DMSO was used as negative control. No inhibition zone is indicated by letter NS (Not Sensitive)

DISCUSSION

Plant composition varies considerably depending on species and seasonal changes in environmental variables including pH, sunlight, mineral availability, and water, and these altering environmental circumstances are factors that induce changes in plant biochemistry and nutrition (De Melo et al. 2021). The chemical makeup and active components of the genus *Rhamnus* have been widely investigated, but this is the first study to look into *R. pentapomica* phytochemical and biological nature. In the present study it was found that the stem bark of *R. pentapomica* contains good quantity of protein. The average protein content in the sample was 8.86%, which is above the critical value of 7.0% as reported by Kafeel et al.(2013). A protein content of the recent study is higher to the finding of Adnan et al.(2010) and it was described that high contents of protein in plant parts are for building and repairing of body tissues, regulation of body processes and formation of enzymes, hormones and antibodies that enable the body to fight infection (Osunlana et al.2018). The deficiency of proteins leads to reduced appetite, low feed intake and poor food efficiency that in turn results in poor growth and development of livestock (Kafeel et al.2013).

In addition, powdered *R. pentapomica* stem bark contain least % ratio of fat content (0.65% DW) which is similar to the finding of Ajayi et al.2018). Those high fat

contents consumptions are the leading step towards heart diseases, atherosclerosis, aging, obesity and cancer (Khan and Khan 2020).Protein, carbohydrates, and fats are the necessary elements of life. The characteristics and amount of proteins present in the plant are major element and important for the selection of plants for nutrition, systematic categorization and plant development programs (Ahmed et al.2013).

Fibres contents of stem bark of *Rhamnus pentapomica* are parallel to the work of Rolls et al.(2005). United States National Academy of Sciences, Institute of Medicine, suggest that adults should consume 20–35 grams of dietary fibber per day because indigestible portion of food in gastrointestinal tract change the nature of the contents of the tract, absorb cholesterol (the risk factor of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer)and provide bulking, thereby easing defecation (Osunlana et al.2018). Fruits and vegetables offered the most rapid methods of providing adequate supplies of vitamins, minerals and fibre to people. They are also food sources with low energy density which are useful in weight management (Rolls et al. 2005).

The relative value of carbohydrates is in line with the work of Erwa et al.(2019*)*.According to Olayinka and Etejere (2018) appropriate dietary components that might be used to supplement a high-carbohydrate diet. Carbohydrates are the principal sources of energy and are an excellent food source. Their direct use in human nutrition or development of balanced diets for animal nutrition is recommended (Amabye 2015). Higher carbohydrate contents in stem bark of *R.pentapomica* are in line with the carbohydrate content in the leaves of *Ficus capensis* previously investigated by Achi et al.(2017).

The moisture contents in stem bark of *R. pentapomica* is estimated at 7.3% which is very important because plant which falls below the 15% moisture contents are said to be a safe storage limit for food materials. Thus plants with lower moisture contents can be stored for long without fear of deterioration (Osunlana et al.2018). Our moisture contents were also in line with the work of Omotayo et al.(2016).

The ash content of about 7.34% indicates that the test plant is rich in mineral elements. Ash is important, because mineral matter may be the cause of a pharmacological effect (Thomas and Krishnakumari2015) and responsible for their use as anti-hypertensives (Aborisade et al.2017). The mean gross energy of the stem bark was highest (151.60 Kcal/100g).The consequence of proximate assessment point out that stem bark of *R. pentapomica* may be used as create substitute source of chief dietary component. The objectives of the present research work were to quantify the proximate composition of nutrients, mineral content in *R. pentapomica* stem bark, determine phytochemicals in various extracted samples and evaluate their antibacterial and anti-fungal action.

Essential minerals, like Ca, K, Mg, and Na and trace elements, like Fe, Mn, Pb, Zn, Cu, and Co, are important for plants metabolic processes, such as chlorophyll synthesis, respiration, as well as protein structure and function and the consumption of plants rich in minerals is associated with numerous health benefits (Ouerfelli et al.

2021). In the present study, it was observed that considerable amount of Calcium, Magnesium, Iron, Potassium, Sodium Cobalt, Zinc, Lead, Manganese and Cupper exist in plant sample. The concentration of essential elements, Pb, Mn, Co, Cu and Zn seem to be minor which is near to the safety bounds according to Shahbazi et al. (2016). The reduced concentration of Cu, Zn and Fe is a sign of less or no noxiousness of the stem bark of the *R. pentapomica* as heavy metals are the causal agents of tumour, kidney and liver complications (Achi et al. 2017).Calcium being a co-factor in some enzyme catalysis, it is also essential for blood clotting, bone and teeth formation and magnesium is required in the plasma and extracellular fluid of human body for maintaining osmotic equilibrium (Achi et al.2017). Megnesium also required in the activation of enzyme pancreatic lipase (Saraf et al.2013) and low serum and dietary Mg maybe related to the etiologies of cardiovascular problems, hypertension, diabetes, and atherosclerosis in humans (Anal and Chase 2016). Iron essential for haemoglobin formation and plays a role in energy transfer within the plant and also an essential constituent of certain enzymes and proteins which facilitates the oxidation of biomolecules to control obesity (Achi et al.2017). Iron deficiency results in anaemia, adverse pregnancy outcomes, developmental delays and impaired physical work performance (Aparna and Pooja2016). Moderate quantities of potassium and sodium were present in the stem bark of *Rhamnus pentapomica.* According to Achi et al.(2017) Potassium is essential elements and is required in large amounts for proper growth and plant reproduction. Besides, potassium and sodium work as cations of extracellular and intra-cellular fluids and aid in maintaining electrolyte balance in the body. The minimum daily intake of K and Na are 3.5 and 2.4 g and below the required intake may lead to body weight loss and nerve disorder (Aparna and Pooja2016). Minerals found in stem bark of *R. pentapomica* to be present in trace quantities are Co, Zn, Pb, Mn and Cu. Their high amounts are not ideal and not desirable for the functioning of the body (Achi et al. 2017). Cobalt (Co) being a trace element it is very necessary in all mammals for the normal functions of the body and in human it is used to treat several different types of cancer and anaemia but the intake of high amount can cause heart diseases (Haq et al.2012). Moderate % of Zinc is vital in transcription, translation, cellular differentiation and immunity and tonic (Achi et al.2017). Lead (Pb) is toxic metal and non-essential element for human body as it causes a rise in blood pressure, kidney damage, miscarriages and subtle abortion, brain damage, declined

fertility of men through sperm damage, diminished learning abilities of children and disruption of nervous systems (Haq et al.2012). The Mn is also trace element required for normal growth, development and cellular homeostasis and it is correlated with many therapeutic properties and useful against diabetic and cardiovascular diseases, have role in neurodegenerative diseases (Anal and Chase 2016) while Copper (Cu) play important role in treatment of chest wounds and prevent inflammation in arthritis and similar diseases (Haq et al.2012).

The phytochemical screening of *Rhamnus pentapomica* stem bark MERP extract, CFRP, EFRP, and BFRP fractions revealed that this plant is a rich source of alkaloids, phenol, flavonoids, saponine, and steroids. Many studies have found different phytoconstituents in therapeutic plants, and our findings are consistent with their findings. Flavonoids, saponins, terpenoids, tannins, and phenols were previously identified in *Zizyphus xylopyrus* (Raghavendra et al.2015), phenols and flavonids in *Rhamnus cathartica* (Hemadri et al.2015), and flavonoids, tannins, and saponins, cyclopeptide alkaloids, and polysaccharides in *Zizypus* (Getahun et al. 2012). Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids which further synthesis complex chemical substances (Hasan et al.2019) and are very essential for humans due to their therapeutic properties (Das and Gezici2018). Terpenes are traditionally used as carminative, stimulant, and counter irritant, anti-rheumatic, perfuming and flavouring agents (Panda and Kar1997). Glycosides have vast therapeutic efficacy like laxative, purgative, ant rheumatic, demulcent, cardio tonic, *etc*.). Phenols and flavonoids have an antioxidant, anti-allergic, antibacterial, anti-inflammatory, antimicrobial and anticancer activity (Shakya2016). Flavonoids possess antioxidant activity, anticancer; reduce risk of coronary heart diseases, acts as phytoestrogens (Samanta et al.2011) antiallergic, anti-inflammatory, antioxidant, antimicrobial, antiviral, anticancer, *etc*. (Ravishankar et al.2013). Pharmacological actions of alkaloids are poisons, stimulant, astringents, anti-inflammatory, antihypertensive, analgesics, expectorants, *etc*.(Shakya,2016). Saponins show anti-inflammatory, antihepatotonic, wound healing, veinotonic, expectorant, spasmolytic, hypoglycemic, antimicrobial and antiviral (Onike2010). The present work on stem bark of *R. pentapomica* would provide helps to the academics for further work like isolation, identification, characterization and their therapeutic effect on more unexplored diseases and can be used as a cheap and safe drugs and medicine in future.

In addition to their phytochemical profile, *R. pentapomica* stem bark extracts exhibited good antibacterial activity against all Gram positive and Gram negative bacteria strains, as evidenced by their sensitivity and zone of inhibition at dosages ranging from 1 to 3 mg ul-1. One of the most serious dangers to the food business and consumer health is foodborne disease caused by bacteria or their toxins, viruses, parasites, or other unexpected agents (Ouerfelli et al.2021).

The antimicrobial investigation indicated that the MERP extract, CFRP, EFRP, and BFRP fractions of *R. pentapomica* stem bark suppressed the growth of *Staphylococcus aureus* and *Bacilus subtilis*. *Bacillus atropheus, Pseudomonas aeruginosa, Salmonella typhi,* and *Klebsillea pneumonia* were reported to be sensitive to MERP, CFRP, and EFRP fractions. The BFRP fraction, on the other hand, showed no inhibition against *Klebsillea pneumonia*. The MERP extract, EFRP fraction, and BFRP fraction were all highly efficient against *E. coli*. The CFRP fraction, though, has no antibacterial activity against *E. coli*. Study on stem bark of *Rhamnus pentapomica* approves that due to the presence of phenolic content, it has been recognised to have various biological potentials including anti-bacterial and anti-fungal activities (Abd-Alrahman et al.2013).Polyphenols have a lot of antibacterial properties. The capacity of extracellular proteins to interact with and form complexes with the bacterial cell membrane is most likely the driving force underlying their activities. They have also been recommended as a therapy for fungal infections in people because to their ability to suppress photogenic spore development in plants(Ouerfelli et al. (2021). Though, the results noticeably insist on that the antibacterial activity show a discrepancy with the bacterial strain used, and the solvents used for extraction. It is to be expected that the potential of medicinal plants against various human and plants pathogenic bacteria and fungi detected in different solvent extracted samples of *Rhamnus pentapomica* is due to the collective effects of all or most of the secondary metabolites present in each extract by playing their role against pathogens via several different mechanism and decreasing the chance of resistivity (Carranza et al.2015). Furthermore, the lack of antimicrobial action in CFRP against *E. coli* and BFRP against *K. pneumonia* strains might be attributed by the acquisition of resistance mechanisms in those strains or the concentration of *R. pentapomica* extracts being insufficient to suppress bacterial growth.

According to the findings, the MERP extract, CFRP, EFRP, and BFRP fractions of *Rhamnous pentapomica* stem bark had a high antifungal effect against *Fusarium oxysporum*. Best antifungal potential was recorded for MERP extract, CFRP, and EFRP fractions against *Candida albicans* and *Aspergillus niger*. CFRP and BFRP fractions also inhibited growth of *Pythium debaryanum*. The CFRP fraction exhibited the best antifungal efficacy against *Aspergilus flavus, Alternaria alternative*, and *Alternaria solani*. In contrast, the BFRP fraction exhibited no antifungal activity against *Candida albicans* or *Aspergillus niger*, while the MERP extract and EFRP fraction had no antifungal activity against *Pythium debaryanum*. Antifungal activity against *Aspergillus flavus,*

Alterneria alternata, and *Alterneria solani* was also not observed in the MERP extract, EFRP, or BFRP fractions.The presence of fatty acid in phytochemical profile is the confirmation for antifungal activity of *R. pentapomica* and due to this potential it can be used as natural environment friendly fungi-toxicant against *F. oxysporum* and other species(Aftab et al.2019).It had been observed that the higher antifungal potential of any extract is also due to the presence of phenols and flavonoids in that extract (Banaras et al.2017).Phenol and ester have also been shown to have antimicrobial effects (Gokul and Priya2019).

CONCLUSION

The findings suggest that *Rhamnus pentapomica* is a good source of proximate nutrients with appropriate water and ash contents, revealing nutritive value (151.60k cal/100 g). The richness in minerals content, and secondary metabolites, as well as their unequal distribution in its MERP extract, CFRP, EFRP, and BFRP fractions impart strong antibacterial and antifungal properties, which has led to their use in pharmaceutical, cosmetic, and food products to improve quality while ensuring consumer safety.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

Yaseen Ur Rehman and Arshad Iqbal contributed to the study conception, design, conceptualization, methodology, investigation and data analysis. The first draft of the manuscript was written by Yaseen Ur Rehman helped by Muhammad Shuaib, Material preparation, data collection and analysis were performed by Yaseen Ur Rehman and Arshad Iqbal supervised and validated the work by Muhammad Shuaib. All authors commented on previous versions of the manuscript, read and approved the final manuscript.

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