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Evaluation of medicinal properties, phytochemical and nutritive attributes of different parts of wild *Ziziphus nummularia* (Burm. F.) from cholistan desert

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Abstract
Wild plants in arid Rangelands are of great importance as not only a source of livestock forage and land covers but also uses for ethnobotanical and pharmaceutical uses. The present study was conducted for evaluating different parts of wild *Ziziphus nummularia* nutritive and various medicinal attributes. Antimicrobial and antibacterial activities were also studied. Fruits and leaves were collected from Cholistan desert. Results revealed that the antioxidants like Flavonoids, alkaloid, and phenolic contents were found both in fruits and leaves. Fruits of the plant showed higher phytochemicals concentration and nutrient concentrations as compared to leaves. Leaves showed more ash and carbohydrate contents, and better antimicrobial activities were observed in the mixture of different solvents extracts of leaves against gram positive and gram negative bacterial strains (*Bascilus subtillis* and *Pseudomonas aeruginosa*). Antifungal activities were also observed in fungus species (*Aspergillus niger*, *Fusarium oxysporum* and *Penicillium glabrum*) and found statistically significant.

Keywords: jangli ber, jharber, biochemical, secondary metabolites, phytochemicals

1. Introduction
Zizihus nummularia is useful wild plant of arid climate with a high value as edibles of ripe fruits, livestock forage source (leaves), living fence, furniture and building wood, medicinal and fuelwood (Pandey *et at.*, 2010; Azhar, 2014) [13, 2]. It is a thorny Rhamnaceous family shrub and is known locally as *Jangli ber* or *Mallah*. A quick grower, multi-stalked shrub is native to India, Pakistan and Iran (about 2-4 m in height). It has an extensive lateral root system of coppice shoots and root suckers. With wavy-tooth margins, leaves are green, orbicular, rounded simple. Its sand dunes stabilizing ability, soil protection and livelihood source of rural dwellers is not fully understood. It is equally important as medicinal and ethnobotanical shrub in Pakistan (Azhar, 2014) [2]. Leaves are forage to livestock throughout the summer, especially antelope feed on it in addition to grazing. It is normal practice of feed-deficient regions of Punjab, Haryana, and Rajasthan (Saroj and Awasthi, 2004) [17]. It mainly grazed during early tender stage, but after maturation, thorn creates grazing resistant and only browsed by camels, goats and sheep. Leaves fall in the winter and starts fruiting. These leaves are harvested, sun-dried, processed for future needs, but often deliberately harvested (twice a year) and marketed locally (Pandey *et at.*, 2010) [13]. Ripened fruits are picked by females and children for marketing to some extent in urban and rural bazars. Ripened fruits are highly nutritive and curative in properties. Hence, is used both in many medicines and food preparations. These Fruits are smaller, round, sweet-sour, and far lower than those of *Z. mauritiana* (Rathore, 2009) [16]. In Rajasthani areas of India, ripe fruits are worship items used in religious ceremonials (Purohit and Wajid, 1981) [15]. Dry Fruits are eaten directly or by combining with sesame seeds by making a spicy paste. Another tasty recipe from the Fruit paste is called 'Borakuti' and is high in vitamin C (Rathore, 2009) [16]. An alternative source of revenue for locals is the selling of Fruits. The wood is not a fine timber and is used in the manufacture of handles for various tools, bent-wood seats, beams, fencing posts, supportive walking sticks, small cabinets, windows, doors and fuel and charcoal (Pandey *et at.*, 2010) [13]. This plant is commonly used in Turkish folk medicine as a potent sedative. The fruit is acerbic and is used for cleaning teeth as brush (Shwetajain *et at.*, 2011) [18]. In traditional remedies, various disorders like liver related, obesity, immunity stimulant, fatigue, wounds and insomnia are treated by it. All parts of this plant are usefull. In study pathogens i.e. *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes* and *Candida albicans* were used. Because of its rise in susceptibility to antibiotics, skin and wound problems, urinary tract infections (UTI), gastrointestinal tract and food poisoning are commonly triggered by *S. aureus*, whereas organ infections include influenza, osteomyelitis, endocarditis, phlebitis and meningitis. Infections from and on medical instruments include cardiovascular devices, joint prostheses, and artificial heart valves (Catrina *et at.*, 2018; Khan *et at.*, 2019) [6, 9]. The *E. Coli* commonly triggers infections of the urinary tract (Muzaffar *et at.*, 2018) [10]. *Pseudomonas aeruginosa* is aomnipresent gram-negative, non-glucose fermenter causes a wide range of diseases such as urinary, burning, cardiac, and septicemia with high susceptibility to antibiotics in clinical samples (Narendra *et at.*, 2012) [12].
A community of gram-positive Streptococcus pyogenes pathogenic bacteria produces a broad range of clinical forms. It extends from pharyngitis to necrotizing fasciitis and acute invasive infections. *S. pyogenes* has severe infection ranges from 10%–30% and causes deaths of atleast 0.6 million persons per year. Two major types of infections, superficial infections and systemic infections in humans are caused by *C. albicans* (Patil & Wanjare, 2017) [14]. The study areas of conventional medicinal plants are

antimicrobial, phytochemical, biochemical and clinical studies that are important to sustain our efforts in the quest for new medicines.

At the completion of any ethno-pharmacological research, we expected to validate a conventional medication or to look for a new drug (Khan *et at.*, 2019) [9]. The antibacterial activity of different extracts of Z. nummularia against the growth of selected Gram positive and negative bacteria and fungi (*C. albicans*) and their phytochemical properties have been studied.

Material and methods

Plant collection and sample preparation

Plant parts were collected from Cholistan desert rangeland from different seasons for picking mature Fruits and washed with distilled water, weighed fresh, shade dried and further oven dried at 40ºc for 24 hours for removing moisture percentage before grinding.

Samples in coarse powder form were placed in air tight containers for biochemical and nutritional analysis.

Extract yield and Preparation

The dried extract yield was determined by using the equation as

$$\text{Yield (g/100g)} = (W_1 \times 100) / W_2$$

W_1 = extract weight after solvent evaporation
W_2 = Dry plant material weight

Aqueous Extraction: 25g oven-dried powder of leaves and Fruit each was boiled in 500 ml distilled water and allowed to evaporate till one fourth remained (separately). Then filtered and centrifuged for 20 min at 5000 rpm and kept at 4°C.

Organic solvent Extraction: 25g oven-dried powder of both parts dissolved in 250ml of ethanol, methanol, chloroform and water separately. Both mixtures were kept in shaker for 24 hr at 150 rpm. After filtration, mixtures were allowed to evaporate for concentration. From these dried extracts other concentrations were prepared.

Growth medium: Nutrient Broth/Nutrient Agar Medium (NBM/NAM) is used for the growth of bacteria and Potato Dextrose Agar Medium (PDAM) was used for the growth of fungal species.

Preparation of Reagents: Nutrient Agar (2.8%), Sabouraud Dextrose Agar (6.5%), Nutrient Broth (1.3%), Potato Dextrose Agar (3.9%) reagents was prepared for the growth of bacterial and fungal species according to standard methods.

Preparation of Test Plates for Antimicrobial Screening Tests: 10ml of the medium was poured in test plates (NA and PDA) and kept at 37°C for 24 hr.

Isolation of microorganisms: Microorganisms selected for this study were collected from pathological lab and inoculated on nutrient plates.

Preparation of Sterile Paper Discs: Paper discs were prepared by means of whole puncher, sterilized at 15 lbs for 15 min as described by Javid *et at.* (2017) [8] & Khan *et at.* (2019) [9].

Chemical analysis

The preserved grinded samples of both parts were analyzed by using standard techniques for Dry matter (DM), ether extract fat (EEF), crude protein (CP), crude fiber (CF), total carbohydrates and total ash and as adopted. Phytochemicals analysis for total alkaloids, flavonoides and total phenolics were estimated by aluminium chloride (AlCl3) colorimetric and determined by Folin Ciocalteu's Reagent method. A detail of these methods is described in our previous publications (Azhar, 2014; Azhar *et at.*, 2014; Azhar, *et at.*, 2015; Azhar, *et at.*, 2020) [2, 3, 4, 5].

Statistical analysis

Collected results were statistically analyzed through Analysis of variance (ANOVA) technique on Statistix 8.1 software. For the comparisons of obtained results HSD Tuckey test was used in Randomized Complete Block to discuss the nutritive and medicinal parameters.

Result and Discussion

The nutrient concentrations of CF is 21.053% in Fruits of *Z. nummularia*) and lower in leaves 17.843%. % Ash, protein, NFE contents were maximum in leaves i.e., 23.992%, 28.072%, 49.95%. EEF concentration, N concentration, P and concentration of K were alsmost similar in both parts (table 1). These findings suggest that all medicinal shrubs have adequate amounts of all necessary food nutrients and be a good livestock feed. Current findings are in accordance with previous researches that leaves of *Z. nummularia* are 17.99% crude fibre (CF), 3% ether extract (EE), 14% crude protein (crude protein), 55% nitrogen-free extract (NFE), 72% carbohydrates, 11% ash, 0.15% phosphorus, 2.9% calcium (Nath *et at.*, 1996) [11]. Similarly, Azim *et at.* (2011) [1] reported, the % of CF, Ash, DM, CP, NFE and EE in leaves was in rang 20.21%, 7.93%, 23.28%, 11.48%, 58.94%, and 1.44% respectively. Chemical composition of dry Fruits of *Z. nummularia* have Ash (1.68 g /100 g) moisture, (11.01 g /100 g) crude fat, (0.89 g /100 g) protein, (51.01 g /100 g) carbohydrate, (1703.21 ppm) potasium (K) and (52.99 ppm) and calcium (c)(Gupta *et at.*, 2011) [7].

Table 1: Nutritive parameters

Plant	Plant parts	Nutritive value %							
		N	Protein	CF	Ash	K	EEF	P	NFE
Z. nummularia	Fruit	3.53A	22.385B	21.053A	26.711B	0.82A	4.27A	0.0675A	47.14B
	leaves	3.927A	23.992A	17.843B	28.072A	0.77A	4.7A	0.0516A	49.954A

N (Nitrogen), P (phosphorus), K (potassium), CP (crude protein), CF (crude fiber), EEF (ether extractable fat), NFE (Nitrogen free extract)

Secondary metabolite Composition

The phenolic contents concentrations were about similar (4.14 & 4.01 mg g-1 DW) in Fruit and leaves of *Z. nummularia* (table 2). Chemical study of plant parts showed

a noticeable heterogeneity in the compositions among both components. This difference possibly attributed to the differing features of different parts of plant. It is not surprising, however, that the nutritional benefit, the palatability, the domestic and commercial usefulness of this plant is obvious.

Table 2: Composition of Secondary metabolite

Plant name	Plant part	Phenolic (mg/.o1gm)		Flavonoids (mg/.o1gm)		Alkaloids (gm/.5gm)	
		Mean	SE	Mean	SE	Mean	SE
Z. nummularia	Fruit	4.14	0.04	0.10	0.02	0.05	0.01
	Leaves	4.01	0.07	0.06	0.01	0.03	0.01

Antimicrobial activity

Antimicrobial activities of leaves and Fruit extracts of Z. nummularia against pathogenic strain of bacteria are described below.

Against *Escherichia coli*

Results in table 3 revealed that leaves samples in ethanol and methanol extract showed different potential against *E. coli* with ZOI 10.34±0.33mm and 9.23±0.33mm separately. Leaves in choloroform extracts showed different potential against *E. coli* with ZOI 9.66±0.31mm. Similarly, leave samples in water extract showed weaker antimicrobial activity against *E. coli* with ZOI 9.23±0.29mm. In ethanol extracts, the maximum antimicrobial activity was observed.

Z. nummularia fruit in ethanol extract showed different potential against *E. coli* with ZOI 11.65±0.28mm and in methanol extract showed different potential against *E. coli* with ZOI, 10.61±0.31mm. Fruit in chloroform extract showed different potential against *E. coli* with ZOI, 9.34±0.32 mm and samples in water extract showed potential against *E. coli* with ZOI 8.42±0.35mm.

Against *Pseudomonas aeruginosa*

Leaves ethanol extract seen the highest sensitivity to *Pseudomonas aeruginosa* (12.03±0.33mm) in comparison to ZOI in other solvent like Methanol (11.59±0.33), chloroform (9.69±0.30) and water (9.15±0.33).

Antibacterial activity of Fruit extracts in ethanol was observed maximum (12.33±0.32mm). In methanol extracts the significant value against *pseudomonas* was with ZOI 11.30±0.28mm. Fruit extracts in chloroform was much less against *P. aeruginosa* i.e. the ZOI was 9.99±0.32 mm (table 3).

Against *Staphylococcus aureus*

The extracts of *Z. nummularia* (leaves) in ethanol exhibited highest potential against *S. aureus* with 8.03±0.32mm ZOI. While in methanol showed similar results with ZOI 8.02±0.30mm against *S. aureus*. The highest susceptibleness was measured in extracts in water with maximum ZOI 7.75±0.24mm.The minimum potential was measured in extracts of Fruits in ethanol and methanol with ZOI 7.65±0.29mm and 6.99±0.33mm.

Against *Streptococcus pyogenes*

The minimum ZOI of leaves extracts in methanol was 7.80±0.38mm against *S. pyogenes*. Chloroform extract of leaves exhibited no antimicrobial activity against S. *pyogenes*. Significant antimicrobial activity was seen in the chloroform extracts of leaves (7.74±0.34 ZOI) least in ethanol and water extract with ZOI 6.99 mm against fungal strains.

Against *Candida albicans*

Leaves extract of ethanol exhibited highest antibacterial activity (7.35±0.26mm). No results were observed in Chloroform and water extracts. Fruit extract in ethanol showed minimum ZOI 8.22±0.33mm. Similarity was observed in methanol and chloroform extracts with maximum ZOI 8.01±0.36mm

Table 3: Antimicrobial activity of *Z. nummularia* against pathogenic strains

		Ethanol	**Methanol**	**Chloroform**	**Water**
E.coli	Leaf	10.34±0.33	9.23±0.33	9.66±0.31	9.23 ±0.29
	Fruit	11.65±0.28	10.61±0.31	9.34±0.32	8.42± 0.35
P.aeruginosa		Ethanol	Methanol	Chloroform	Water
	Leaf	12.03±0.33	11.59±0.33	9.69±0.33	9.15± 0.33
	Fruit	12.33±0.32	11.30±0.28	9.99±0.32	8.58± 0.33
S. aureus		Ethanol	Methanol	Chloroform	Water
	Leaf	8.03± 0.32	8.02±0.30	-	7.75±0.24
S. pyogenes	Fruit	7.65± 0.29	6.99 ±0.33	7.33±0.32	7.34±0.33
		Ethanol	Methanol	Chloroform	water
	Leaf	7.74±0.34	7.80±0.38	-	6.99±0.24
Candida albicans	Fruit	7.30±0.29	7.34±0.32	-	7.00±0.40
		Ethanol	Methanol	Chloroform	water
	Leaf	7.35±0.26	7.33±0.31	-	-
	Fruit	8.22±0.33	8.01±0.36	8.01±0.34	-

Antifungal Activity

Antifungal assay of leaves and fruit extracts of *Z. nummularia* was carried out against four different fungal species including *Aspergillus flavus*, *Aspergillus Niger*, *Fusarium oxysporum* and *Penicillium glabrum*.

The results indicated significant antifungal activities in leaves concentrated samples while diluted sample showed negative control of antifungal activity.

Fruits showed positive control in concentrated and diluted samples.

Table 4: Antifungal activity of leaves and fruit extracts against fungal spores

Fungal growth inhibition						
Plant extracts			Fungal Species			
			A. flavus	A. niger	F. oxysporum	P. glabrum
Chenopodium album	Leaves	Conc.	+	+	+	+
		Dil.	-	+	+	+
	Fruit	Conc.	+	+	+	+
		Dil.	+	+	+	+
Avena fatuda	Leaves	Conc.	+	-	+	+
		Dil.	+	+	-	-
	Fruits	Conc.	+	+	-	+
		Dil.	+	+	-	+
Fungicide			+	+	+	+
Water			+	+	+	+

Conclusions

Zizyphus species are grown naturally as wild in dry areas due to their ecological requirements and are potential as edible fruits, livestock feed (leaves), branches as fence, stem as furniture wood, construction material, fuel wood and also in folk medicine. Its leaves and fruits are suitable in nutraceutical products as leaves contain crude protein, crude fibre, total minerals and starch etc. It could be excellent firewood and chemical properties of *Z. nummularia* leaves and fruits having proteins (amino acids), flavonoids, alkaloids, saponins, phenolic compounds have various beneficial health effects due to antioxidant. It showed antibacterial activity against bacterial strains *S. aureus* and *E. coli*. Could be helpful for treating of various ailments. The plant leaves and fruit extract of *Z. nummularia* showed a considerable antimicrobial activity against selected pathogens and antibacterial activities against bacterial strains. Phytochemical attributes confirmed *Z. nummularia* as a rich bioactive source.

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