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Phytochemical Screening and Antioxidant Activity Of Stem Extracts of *Zizyphus oxyphylla* Edgew

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Abstract: Keeping in view the folkloric history, stem extract of *Zizyphus oxyphylla* Edgew (Rhamnaceae) was examined phytochemically and for scavenging activity. Results of qualitative phytochemical analysis confirmed the presence of alkaloids, terpenoids, tannins, saponins, coumarins and flavonoid. Total phenolic contents (TPC), Total flavonoid contents (TFC) were evaluated spectrophotometrically. Maximum phenolic content was recorded in aqueous extract 171.2±7.13 mg/g GAE followed by n-butanol and crude extract fraction with 159.6±5.13mg/g and 158.3±6.25mg/g GAE respectively, TFC analysis has also shown significant results in aqueous extract 94.1±3.70 mg/g QE followed by crude extract and n-butanol extract having close readings of 89.0±3.40 mg/g and 88.0±4.72mg/g QE correspondingly. DPPH assay has revealed worth mentioning scavenging activity in aqueous extract; 1.93±0.04 µmol/g extract TE followed by n-butanol, crude extract and ethyl acetate exposed close readings 1.88±0.06 µmol/g, 1.84±0.07 µmol/g and 1.67±0.04 µmol/g TE respectively.

Key words: *Zizipus oxyphylla* · Stem · Phytochemical screening · Antioxidant activity (DPPH)

INTRODUCTION

Plant is a factory of medicine and its history can be justifiably compared with human civilization. It is a top most good source of drugs for human wellbeing's long life, smartness with good health because plants carry search qualities in it. The medicines which we hand locally are inherited medicines duly floated to the today's world which got the status up to the minutes with the passage of time. From the start of Islam, Arab scholars had keen interest in the use of herbal medicines as compared to other meritorious civilizations. It is an admitted fact that base of modern medicinal science floated to Europe from Islamic civilization. If compared to Synthetic medicines, natural compounds carry little side effects. The role of aforesaid plants as medicines, got roots for their chemical analysis which resulted in isolation of bio-active natural compounds [1].

The genus *Zizyphus* belongs to family *Rhamnaceae*, comprising of about 100 species throughout the world. Among those only 6 species are home-grown in Pakistan listed down, however the genus is broad-spread in Indo Malaysia, Mediterranean regions, India, Tropic America and Pakistan. In Pakistan this specie *Zizyphus oxyphylla* is distributed in Buner, Hazara, Swat and Gari Habibullah [2].It is traditionally used for treatment of jaundice, pain, inflammation and fever. Leaves on chewing completely anaesthetize the taste, while fruit is used as emollient and useful expectorant in bronchitis [3]. Keeping in mind the medicinal importance of *Zizipus oxyphylla* the study was planned to explore the phytochemistry of the said plant. The objective of the study was to explore the photochemistry of the said plant, for this purpose the stem was selected and phytochemical screening and antioxidant activity was evaluated.

MATERIALS AND METHODS

Collection of Plant: *Zizyphus oxyphylla* Edgew was collected from Northern Areas of Pakistan (Swat) in February 2011 and identified by Botany department, Peshawar University. It was brought to the Laboratory in

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KPK Agriculture University Peshawar, Where it was stored for drying purpose at room temperature. After which it was subjected to Extraction and further analysis.

Extraction: The plant material (stem) was collected during the appropriate season, dried under suitable but temperate conditions and later on pulverized for further chemical treatment. The powder plant material (about 8 kg) was macerated thrice with methanol for 7 days for primary extraction. The filtrate was evaporated using rotary evaporator which has given about 375g of crude extract which was then subjected to phytochemical analysis [4].

Fractionation of Crude Extract: After getting 355g crude extract of the plant, it was firstly treated with distilled water and then aqueous fraction was treated with n-hexane, chloroform, ethyl acetate and n-butanol sequentially. N-hexane has extracted about 42.5g fraction followed by chloroform, ethyl acetate and n-butanol that is 11g, 37.3g and 163.5g, respectively. The remaining aqueous fraction was about 97.1 gram.

Phytochemical Screening of *Zizipus Oxypylla* Stem: Chemical screening of *Zizipus oxypylla* stem were perform in chloroform, ethyl acetate, aqueous extract and n-butanol followed by subsequent standard qualitative protocols given by Aayola *et al.* [5] for phytochemicals. The methods for each test are given below accordingly.
Alkaloids Initially alkaloids were extracted from acid-base crude extraction and chloroform extracts subsequently. After dividing them into two fractions, each of them is subjected to different tests. Draggendorff's reagent and Mayer's reagent were added in to each fraction. The presence of Alkaloids was confirmed with cream formation in Mayer's test while reddish-brown ppt. in Draggendorff's test.

Flavonoids: A few drops of sodium hydroxide were added in the extract solution which has shown an intense yellow color which was again vanished by adding few drops of acetic acid, therefore confirmed the presence of flavonoids.

Terpenoids: For detection of terpenoids we treat our sample with petroleum ether and extract it against chloroform. Treating the extracted layer with acetyl anhydride and HCl turns pink to purple shows positive sign for terpenoids.

Tannins: 0.5 g of crude extract was boiled in a test tube with 10 ml of water and after boiling we had filter it. By adding only some drops of 0.1 % $FeCl_2$ solution, Brownish-green or bluish-black color formation was the evidence for the existence of tannins.

Saponins: 10 ml water (distilled) was taken in a test tube and 0.5 g of crude extract was added into it followed by shaking. An emulsion was formed after froth formation when it was mixed with 2-3 drops of olive oil and shaken strongly. It was the confirmation point for presence of saponins.

Coumarins: For checking the presence of Coumarins, a filter paper moistened in sodium hydroxide kept over the mouth of a test tube, in which plant extract is present in boiling form. After removing filter paper if it has shown yellow fluorescence under ultra violet light so it was an indication for presence of Coumarins.

Total Phenolic Contents (TPC): Extract of *Zizyphus oxyphylla*was checked for total phenolic content by Folin-Ciocalteu reagent, a protocol represented by (Li *et al.*, 2008) [6] with minor modifications. Initially dilutions of crude extract were done about 50 times with water (deionnized) preceding to study. 1 ml of diluted F-C reagent (diluted with deionized water 10 times) was mixed with 1 ml of extract (diluted). After this, incubation of the mixture was done for 5 minutes at room temperature. 0.8 ml of 7.5% $NaCO_3$ (anhydrous) was added into that mixture followed by immediate mixing for 10 s on vortex mixture and finally incubated for 2 hours in a dark environment. A blank sample was assessed with the same procedure having deionized water instead of *Z. oxyphylla.* Absorbance was determined by comparison with blank at 765 nm under ultraviolet light spectrophotometer. Standard curve calibration was done through Gallic acid. Triplicate random crude extract samples were analyzed and result was mentioned in mg Gallic acid equivalent/g of sample DW.

Total Flavonoid Contents (TFC): According to Ozsoy *et al.* (2007) [7] crude extract and other fractions of *Z. oxyphylla* were checked for total flavonoid content (TFC). Extract of *Zizyphus oxyphylla* was taken 0.25 ml and initially mixed with 1.25 ml of water (deionized), after which 75 µl of 5% sodium nitrate and after 6 minute 150 µl of 10% Aluminium chloride was added and mixture was

permitted to stand for 5 minutes at room temperature. Afterward 0.5 ml of 1 molar NaOH was added into same mixture. Now 275 µl of water (deionized) was added and mixture was subjected to vortex mixer for 10 s. Absorbance of the extract was recorded spectrophotometrically at 510 nm with UVi light. Blank was run using deionized water instead of *Z. oxyphylla* extract. Standard curve calibration was done with Quercetin. Triplicate samples of plant were analyzed and results were recognized mg of Quercetin equivalent/g of sample DW.

Anti-oxidant Activity (Dpph Radical-scavenging): The protocol used for DPPH anti-oxidant activity was described by (Saha *et al.* 2004) [8]. 100 µl of crude extract and other fractions of *Zizyphus oxyphylla* were treated with 3.9 ml (60 µM) of DPPH (ethanolic) and then instantly subjected to vortex mixer for 1 mint and then it was permitted to stand for 25 to 30 minutes at room temperature. Blank was primed with 100 µl water (deionized) replacing *Zizyphus oxyphylla* extract. Absorbance of the mixture was checked spectrophotometrically at 517 nm against blank with ultraviolet light. At the same time the absorbance of negative control was precisely checked at 517nm and the value was used as constant for further calculation. The percent activity (DPPH scavenging) was calculated as $[1- (A_s / A_c)] \times 100\%$ whereas A_c stands for negative control absorbance and A_s stands for sample absorbance). Standard curve calibration was done using Trolox solution. Triplicate samples were analyzed for precise readings and coming results were expressed as µmol trolox equivalent/g of sample DW.

Statistical Analysis: All The experiments were performed in triplicates. The data was analyzed on SPSS 16.1 Software. The data was arranged as means of Means ±SD (Standard Deviation) in the Tables.

RESULTS

The aim of the study was to investigate the stem crude extract of *Z. oxyphylla* for presence of different classes of phytochemicals and for screening of antioxidant activity. Form the results it has confirmed that the crude extract showed the presence of Alkaloids, Terpenoids, Tanins, Saponins and Flavonoids except coumarines as shown in Table 1.

Table 1: Phytochemical analysis of crude extract of *Zizyphus oxyphylla*(Qualitative)

S.NO	Phytochemical Class	Status
1	Alkaloids	Positive
2	Terpenoids	Positive
3	Tanins	Positive
4	Saponins	Positive
5	Coumarines	Negative
6	Flavonoids	Positive

Table 2: TP content of various extracts from *Zizyphus oxyphylla*. Results are the mean ±SD of three parallel measurements. The values bearing different letters are very significantly different ($P < 0.01$)

Stem Extract	Total phenolic (mg GAE per g extract)
Crude extract	158.3±6.25^c
Chloroform	122.9±3.52^e
Ethyl acetate	144.2±4/72^d
n-Butanol	159.6±5.13^b
Aqueous extract	171.2±7.13^a

Table 3: TF content of various extracts from *Zizyphus oxyphylla*. Results are the mean ±SD of three parallel measurements. The values bearing different letters are very significantly different ($P < 0.01$) Total Flavonoid Content

Stem Extract	Total flavonoids (mg QE per g extract)
Crude extract	89.0±3.40^b
Chloroform	59.2±2.29^e
Ethyl acetate	71.0±1.81^d
n-butanol	88.0±4.72^c
Aqueous Extract	94.1±3.70^a

Table 4: Antioxidant Activity of *Zizipus oxyphyla* in various extracts. Results are the mean ±SD of three parallel measurements. The values bearing different letters are very significantly different ($P < 0.01$)

Stem Extract	Antioxidant activity (TEAC)
Crude extract	1.84±0.07^c
Chloroform	0.94±0.03^e
Ethyl acetate	1.67±0.04^d
n-butanol	1.88±0.06^b
Aqueous extract	1.93±0.04^a

Total Phenolic Content (TPC): The total phenoliccontent (TPC) in the extract was calculated from a regression equation for standard curve for Gallic acid $y = 0.0396x$ ($R^2 = 0.9975$). Total phenolic of all the 5 extracts are demonstrated in Table 2. AE revealed the highest total phenolic content at 171.2±7.13 mgg^{-1} of extract, expressed as Gallic acid equivalents, followed by n-butanol and crude extract having 159.6±5.13mgg^{-1} and 158.3±6.25mgg^{-1} GAE correspondingly. Chloroform and ethyl acetate extract have shown minimum total phenolic content relatively.

Total Flavonoid Content (TFC): The total Flavonoid content (TFC) in the extract was determined from a regression equation for standard curve for Quercetin y = 0.0067x, (R^2 = 0.999). Total flavonoids of all 5 extracts are expressed in Table 3. The highest total flavonoid content was recorded in aqueous 94.1±3.70 mgg^{-1} of extract, articulated as Quercetin equivalents, while the lowest total flavonoid was recorded for chloroform which was 59.2±2.29mgg^{-1}.

Antioxidant Activity (DPPH Assay): The scavenging abilities of various extracts against DPPH radical were examined by comparing with standard curve made by Trolox. Major antioxidant activity was shown by Aqueous; 1.93±0.04 µmol of Trolox equivalents/g of extract, followed by n-butanol, crude extract and ethyl acetate having 1.88±0.06 $\mu molg^{-1}$, 1.84±0.07 $\mu molg^{-1}$ and 1.67±0.04 $\mu molg^{-1}$ TE respectively. Slight antioxidant activity was measured in chloroform extract (Tables 4).

DISCUSSION

The results of the present study confirmed the presence of flavonoids, alkaloids, saponins, tanins, terpeniods and coumarins. The scavenging activity exhibit good results in all the extracts except in chloroform, which was quite low as compared to other extracts. Singh *et al.,* [9] also reported phytochemical screening and antioxidant activity of the genus *Zizipus*(*Zizipus jujuba* and *Zizipus oeniplia)*, the results of has work confirmed the presence of alkaloids, terpenoids, tannins, saponins, coumarins and flavonoid. The scavenging activity was also confirmed form has research work. Esteki *et al.,* 2012 [10] reported antioxidant activity and phytochemical screening of *Ziziphus jujuba* Mill peel is a rich source of many antioxidant compounds. The antioxidant activity of extracts was investigated by DPPH and reducing power assay. The phytochemical content like tannin and Polyphenol were significantly ($p<0.05$) higher in the raw peel (1.67±0.07 and 7.69±0.09 g/100g respectively), while glutathione (GSH) content of cooked peel (125.75±5.04 µM/100g) was significantly ($p<0.05$) higher than raw peel (99.49±8.84 µM/100g). Gupta *et al.*, 2004 [11] investigated the ethyl acetate extract of *Z. jujuba* bark for antisteroidogenic activity and hence fertility in adult female mice. The results showed reduction of wet weight of ovaries significantly. Reversible antifertility activities of crude extracts were found in rat.

CONCLUSION

From the research it was concluded that the *Zizipus oxyphylla* plant consist of many phytochemical, which can be used for the manufacturing of different drugs. As the plant confirmed the presence of phytochemical so further phytochemical studies should bemade to isolate and identify various chemical compounds. Vast research is required to explore potentially bioactive compounds as plant is used locally for different other diseases such as bronchitis.

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REFERENCES

1. Standen, O.D., R.J. Schnitzer and F. Hawking. 1963. Experimental Chemotherapy.Academic Press, New York. 1: 113-114.
2. Nisar, M., B. Adzu, K. Inamullah, A. Bashir, A. Ihsan and A.H. Gilani, 2007. Antinociceptive and Antipyretic Activities of the *Zizyphus oxyphylla* Edgew. Leaves. Phytother. Res., 21: 693-695.
3. Jan, G., K. Altaf, 2009. Ethnobotanical Leaflets, 13: 1029-1041.
4. El-Bakry, A.A., H.A.M. Mostafa and A.A. Eman, 2009. Antioxidant activity of Rumex vesicarius L. At thevegetative stage of growth. Integrative Medicine, 8(2): 35-38.
5. Aayola, G., H. Coker, S. Adesegun, A. Adepoju-Bello, K. Obaweya, E. Ezennia and T. Atangbayila. 2008. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Tropical J. Pharma. Res., 7: 1019.
6. Li, H., C. Wong, K. Cheng and F. Chen, 2008. Antioxidant properties *in vitro* and total phenolic contents in methanol extracts from medicinal plants. Lebensmittel-Wissenschaft und-Technologie, 41(3): 385-390.
7. Ozsoy, N., A. Can, R. Yanardag and N. Akev, 2007. Total flavonoid content and antioxidant activity of *Smilax excelsa* L. leaf extracts. Food Chem., 110(3): 571-583.

8. Saha, K., N.H. Lajis, D.A. Israd, A.S. Hamzah, S. Khozirah, S. Khamis and A. Syahida. 2004. Evaluation of antioxidant and nitric oxide inhibitory activities of selected Malaysian medicinal plants. J. Ethnopharm., 92(2-3): 263-267.
9. Singh, P., T. Mayank, Y. Sanjay and P.D. Shashi, 2014. Comparative Study of the roots of the plants *Ziziphus oenoplia* and *Ziziphus jujube*.J. Pharmacognosy and Phytochemistry, 3(1): 211-217.
10. Esteki, T. and A. Urooj, 2012. Antioxidant components and activity in the peel of *Ziziphu jujuba* Mill. Journal of Pharmacy Research, 5: 5.
11. Gupta, R.B., S. Sharma, J.R. Sharma and R. Goyal, 2004. Study on the physicochemical characters of fruits of some wild and cultivated forms/spp. (*Zizpihus* spp.). Haryana Journal of Horticultural Sciences, 33(3/4): 167-169.