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In vitro antibacterial and anticancer activity of *Ziziphus*

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Abstract
According to Ayurveda there are large numbers of traditional medicinal uses of *Ziziphus* species and the reasons behind the medicinal potential of this plant are because of some allied substances such as ascorbic acid, thiamine, riboflavin and bioflavonoids and pectin-A. Therefore, this plant is a valuable source of phytochemicals, bioactives, vitamins etc. which makes it to be effective in curing or prevention from various diseases Thus the aim of the present work is to screen the different extracts of *Z. jujube* and its different species i.e *Z. nummularia, Z. mauritiana* plant parts such as leaves, fruit and bark for anti-bacterial activity and *in vitro* anticancer activity. Discussed study offers some preliminary information about antibacterial and anticancer potential of *Ziziphus* species. The exciting feature of the study particularly indicates the phytomedicinal properties of fruits of *Ziziphus* species. Further established composition of components is required in appropriate form for therapeutical application.

Keywords: Antibacterial, anticancer, phytochemical, phytomedicine

Introduction
Ziziphus family Rhamnaceae have fruits like drupes or dry is associated with an Arabic word while the ancient Greeks used the word ziziphon for the jujube. The widely domesticated jujubes are *Z. mauritiana* Lam which is also known as the Indian jujube or ber, Rajabadari (Sanskrit); Beri (Punjabi); Ber (Hindi); Ber (Urdu) i.e. *Z. jujuba* Mill (Common jujube). Whereas the wild variety of *Ziziphus* is *Z. nummularia*. The *Z. mauritiana* or *Z. jujuba* species have been cultivated over vast area of the world. The species comprises of shrubs to small or medium sized trees, which have an extensive range of morphologies such as semi deciduous, branched, might be erect, semi-erect or spreading with height from 3-4 to 10-16 m or more, while trees of 20 m are rare. The Fruits are of greenish, yellow or sometimes reddish in color having sweet and acidic pulp. They are glabrous, globose or oval edible drupe, varying significantly in size from (1-) 1.5 (-2) cm diameter, nevertheless some oval varieties can reach upto 5 x 3 cm. [1]. According to Ayurveda, there are large numbers of traditional medicinal uses such as the root of *Z. nummularia* cures coughs, biliousness and headache wile its bark cures boils and is used for the treatment of dysentery and diarrhea. The leaves are also fond to as antipyretic and helpful reducing obesity. The fruit improves digestion, aphrodisiac, laxative and removes biliousness, burning sensations, thirst, vomiting and has been used widely for their good response in the treatment of tuberculosis and blood diseases. It is also explored as an antidote to aconite poisoning and abdominal pain in pregnancy. The seeds are also useful in leucorrhoea and cure eye diseases [2]. The reasons behind the medicinal potential of this plant are because of some allied substances such as ascorbic acid, thiamine, riboflavin and bioflavonoids and pectin-A. *Ziziphus jujuba* fruits are found to be very rich in vitamins C (188 to 544 mg per 100 gm pulp), B1 (thiamine) and B2 (riboflavin). As compared to other edible fruits, one fruit of ber per day would meet the diet requirements for Vitamin C and Vitamin B complex for an adult man as recommended by FAO/WHO [3]. It is also known to have a high Vitamin P (354 to 888 mg per 100 gm pulp) (bioflavonoid) content. Pectin-A chemically contains 2, 3, 6-tri-o-acetyl D lactose units. Pectin has a number of pharmaceutical properties such as binding bile acid, lowering plasma cholesterol and anti diarrhoeal properties [4]. The different species of the genus *Ziziphus* is also reported to have alkaloids, flavonoids, sterols, tannins, saponin, and fatty acids. Important Flavonoids present in *Ziziphus jujuba* are quercetin and epicatechin knon to help in treating various diseases and disorders. Flavones, isoflavones and anthocyanidins are also present in *Ziziphus* species [5]. Because of therapeutical prospective properties of *Ziziphus* species, they are widely used in folk medicine for the

treatment of some diseases. Therefore, this plant is a valuable source of phytochemicals, bioactives, vitamins etc. which makes it to be effective in curing or prevention from various diseases. Thus the aim of the present work is to screen the different extracts of *Z. jujube and its* different species i.e *Z. nummularia, Z. mauritiana* plant parts such as leaves, fruit and bark for anti-bacterial activity and *in vitro* anticancer activity.

Material and Methods

Chemicals, culture and material

The entire chemicals which are used in the experiments were procured from sigma. The cancer cells were obtained by the NCCS (national center for cell sciences) Pune, India.

Collection of plant material and Extract preparation

Ziziphus *jujube, Ziziphus mauritiana* & *Ziziphus nummularia* (Leaves, Bark and Fruit.) were collected and authenticated from Forest Research Institute, Dehradun. The Leaves, Bark and Fruit were washed thoroughly with running tap water to remove dust particle. After washing plant parts the water is removed by keeping it in between two filter papers. After that the plant material is dried in the hot air oven at 30 ˚C for 8 to 10 hour's. When dry the plant material is powdered with the help of mixer grinder. The plant powder is subjected to extraction with Methanol, Aqueous, Chloroform and Hexane [7].

Antibacterial activity

After that the extracts were filtered and test for their antimicrobial activity. Two Bacterial strains were used for this study i.e. *Staphylococcus aureus* & *Escherichia coli* which were obtained from The Himalaya Drug Company Dehradun. Both bacterial strains were maintained in the laboratory on the surface of Nutrient Agar Slant and preserved in the refrigerator at 4 ºC and taken out and brought to room temperature, whenever required for the study. The antimicrobial assay was conducted by agar well diffusion method. The bacterial strains grown on nutrient agar at 37 ºC for 18 hours were suspended in a saline solution (0.85% NaCl) and adjust to 'A turbidity of 0.5 Mac Farland standards (10 CFU/ml.) The suspension was use to inoculate 90mm diameter petriplate. Well (6 mm diameter) were punched with the help of cork borer in the agar and filled with 50µl of different sample the dissolution of the extracts was added by 10-15% (v/v) DMSO (Di Methyl Sulphoxide) which did not effect the growth of microorganism, in accordance with our control experiments. Plates were incubated in bacteriological incubator at 37 ºC for 24 hours. Antimicrobial activities were evaluated by measuring inhibition zone diameters [7].

Anticancer activity

Anticancer activity was elucidated by observing the cell viability, morphological changes and apoptosis induction after the treatment of extract on HeLa cancer cells**.**

Cell Viability assay

The cell viability was assessed by MTT [3-(4, 5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide)] assay, which determines the metabolically active mitochondria of intact cells. The assay was carried out using MTT cell assay kit, following the protocols described by the manufacturer's (HiMedia, India). Briefly cells were seeded in 96-well plates, with 5×10^3 cells/100 µL and incubated for 24 h at 37 ºC. The cells were then treated with different plant parts extract (50 µg/ mL to 300 µg/ mL) and incubated for another 24 h at 37 ºC in a 5% CO2 atmosphere. The assay was performed by the addition of premixed MTT reagent, to a final concentration of 10% of total volume, to culture wells containing various concentrations of the test substance and incubated for further 4 h [8].

Morlological changes after the extract treatment

For Hematoxylen/Eosin staining, cells (20×10^3 cells per well) were placed in DMEM (Dulbecco's Modified Eagle Medium) by using 24-well plates. After treating with the extracts at different concentrations for 24 h period, the medium was removed, the cells washed with distilled water and fixed in ethanol, and stained with Hematoxylin/Eosin. After staining, the cells were observed by light inverted microscope (Nikon) [10]. By this way, cellular and nuclear morphology is shown in cultured cells stained with Hematoxylin/Eosin [8].

Results

Antibacterial assay

The agar plate well diffusion method for extracts were employed for the determination of antibacterial activity, in which the wells are used as a reservoir of the sample dilutions and the standard dilutions. The reservoir containing the sample dilutions were brought into contact with the inoculated medium and after incubation for 18 to 24 h, the diameter of the clear zones of inhibition around the reservoirs was measured with vernier caliper [9]. However, in case of no activity of the sample, no zone of inhibition will develop. The concentration of the extracts employed was 100 mg/ml were screened for their antimicrobial activity against two commonly prevalent human Pathogenic gram negative and gram positive bacteria's particularly *S. aerious ATCC®6538, Escherichia coli ATCC®25922.* The methanol extract of all the three varieties of *Ziziphus* show moderate to good antimicrobial activity against test organisms, while aqueous extract show no remarkable activity, whereas the Hexane extract of fruits also show significant activity against organism. While other extracts of leaves and bark are not showing any such effects (Table 1).

Table 1: Antibacterial activity of *Ziziphus* species

Name of Plant	Part Used	Name of Micro Organism	Methanol	Aqueous	Chloroform	Hexane
Ziziphus jujuba	Fruit	*E. coli*	14mm	-	8mm	10mm
		S. aureus	10mm	-	10mm	-
	Leaves	*E. coli*	8mm		-	
		S.aureus	-	-	-	
	Bark	*E. coli*	-	-	-	-
		S. aureus	-	-	-	-
Ziziphus mauritiana	Fruit	*E. coli*	11mm		10mm	
		S. aureus	8mm	-	12mm	-
	Leaves	*E. coli*	3mm	-	3mm	8mm
		S. aureus	-	-	-	-
	Bark	*E. coli*	-	-	-	-

		S.aureus	-	-	-	-
Ziziphus nummularia	Fruit	*E. Coli*	16mm	-	9mm	12mm
		S.aureus	12mm		7mm	10mm
	Leaves	*E. coli*	-	-	2mm	-
		S.aureus	-	-	2mm	-
	Bark	*E. coli*	-	-	-	-
		S. aureus	-	-	-	-

Anticancer activity

MTT assay was done to detect the cytotoxic effects of methanolic extract of fruit of all the three varieties of *Ziziphus* i.e *Z. nummularia* (F1), *Z. mauritiana* (F2) *and Z. jujube* (F3). As phytochemical profiling of fruits methanolic extract shows the presence of various phytochemical [10]. Thus we have observed that the wild variety of *Ziziphus* is inducing significant cytotoxicity in HeLa cells (cervical carcinoma cells), whereas the *Z. mauritiana* show also cytotoxicity towards cancer cells. However the *Z. Jujube* is inducing some sort of cytotoxicity toward cancer cells as shown in Fig: 1.

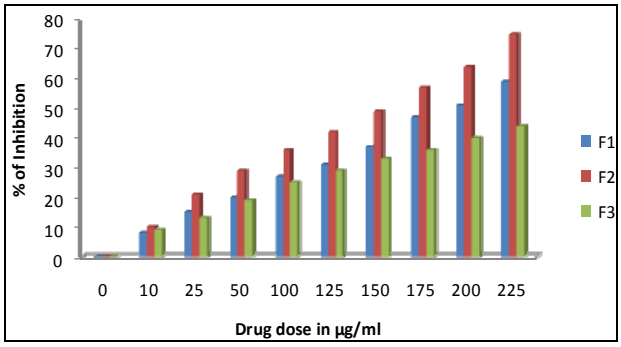

Fig 1: % of Inhibition induced by the extracts

Haematoxylin and Eosin staning

The Staining pictures shows that the sample F2 has got significant cytotoxicity against Hela cells (cervical cancer cell) and after treatment cell density become less and apoptotic changes can be seen such as condensed cytoplasm. F2 also shows cytotoxicity and altered morphology of cancer cells but less then FI. Whereas, F3 is least effective and cytotoxic in comparison to the other extract toward cancer cells. Therefore It is clear by the staining pictures that the order of cytotoxicity is as F2>F1>F3 (Fig 2:).

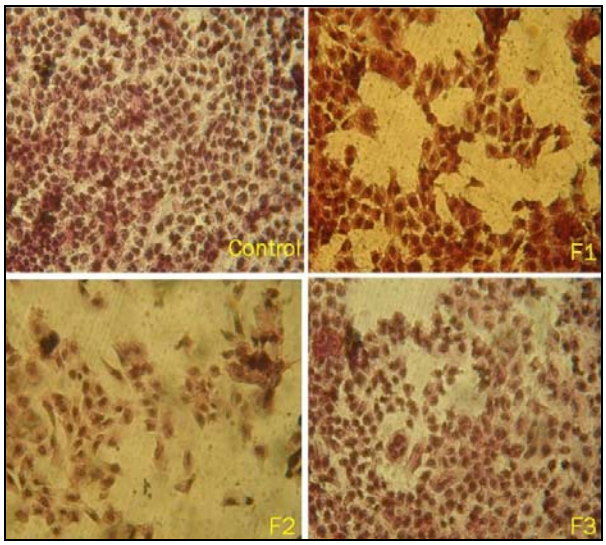

Fig: 2 Hematoxylin and Eosin staining after the treatment of fruit extracts on HeLa cancer cell

Discussion

Z. jujube and its species *Z. mauritiana, Z. nummularia* possesses tremendous medicinal properties, recognized by the presences of secondary metabolites. It has been observed that 16 glycosides and flavonoids, 64 alkaloids and 14 terpenoids are present in this plant. These phyto-chemicals has unique and various therapeutic properties [11]. However they have been ignored as potential prophylactive agents as health products and neutraceutical supplements. While extensive research is also needed to explore the therapeutical potential of *Z. jujube* and its species. Whereas some research studies reported their medicinal potential as antibacterial, antifungal, neuro-protective, anti-allergic, anti-uler, anti-inflammatory and anti-cancer. A study [12] reported the antifungal effects of ethanol extract of the *Z. jujuba* root which showed significant inhibitory activity on fungi *Candida albicans, C. tropicalis, Aspergillus flavus, A. niger* and *Malassezia furfur* (strains 1374 and 1765). Furthermore, extract of root bark of *Z. jujube* exhibited antibacterial activity against 20 bacteria [13]. Furthermore the leaves of *Z. jujuba* also shown to have strong anti-allergic activity [14], measured by its inhibitory effect on hyaluronidase (bovine testes) activation *in vitro.* Whereas a significant feature was found of *Z. jujuba* leaf extract is to stimulate chemo tactic, phagocytic and intracellular killing potency of human neutrophils (infection fighting white blood cells) at 5-50 micro g/ml [15]. Several other pharmacological benefits of *Z. jujube* and its species has been published reporting their neuro-protective [16], chemopreventive [16], Hypotensive and Antinephritic effect [17] and cardio-vascular activity [18] etc. It has been also observed that the Leaf of *Z. mauritiana* have antibacterial activity against several bacteria. However, Ganachari and shiv [19] reported significant and dose-dependent an antiulcer property of *Z. mauritiana* leaf extracts in rats as recognized by its cytoprotective and antisecretory action. A research report also stated that the *Z. mauritiana* leaf extracts possess significant anti-inflammatory activity against carrageenan-induced rat paw edema [20]. The several publishes research studies signifies that this plant is an excellent accessible source of active compounds which can be use to treat the various human ailments through their allied applications. In this study it was demonstrated that extracts of *Ziziphus* species exhibited significant anti-bacterial and cytotoxic activity against HeLa cancer cells. The results of the present study would certainly help to ascertain the anti bacterial and anticancer potency of crude extract obtained from the different parts of *Z. nummularia, Z. mauritiana* and *Z. jujuba* such as fruit, leaves and bark. The interest regarding the research on medicinal plants has increased over the last few decades due to onset of new infection and resistant to several antibiotics to which it was previously susceptible. With the intent of exploring new bioactive compounds from plant origin, we have selected three species of *Ziziphus*, which is locally used as an analgesic, tranquilizer, convulsant and have been prescribed for the treatment of insomnia and anxiety [21]. The results of antibacterial activity of the various crude extracts of *Ziziphus* species are shown in Table 1. Flavonoids from *Ziziphus jujuba* help a lot to treat various diseases and disorders like cancer

and diarrhea. Important flavonoids present in *Ziziphus jujuba* are quercetin and epicatechin. Flavones, isoflavones and anthocyanidins are also present in *Ziziphus* species. After the observation of the significant antibacterial activity of fruits of all the three varieties the Ziziphus species methanolic extracts, we have checked the anticancer activity of extracts on HeLa cancer cells. We have found that the *Z. mauritiana* significantly induces cytotoxicity and altered morphology of cancer cells whereas the *Z. nummularia* and *Z. jujuba* also shows some sort of cytotoxic effects against HeLa cancer cells.

Conclusion

The results of the present study would certainly help to ascertain the pharmacological potency of the crude extracts of *Ziziphus* species. Discussed study offers some preliminary information about antibacterial and anticancer potential. The exciting feature of the study particularly indicates the phytomedicinal properties of fruits of *Ziziphus* species but to develop useful more work is required to further establish composition of components involved and how to isolate them in appropriate form for particular application.

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