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Evaluation of Phytochemical, antioxidant, antibacterial and anti-cancerous activity of *Ficus auriculata* Lour. and *Osyris wightiana* Wall. ex Wight

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ABSTRACT
The present study deals with the phytochemical, antioxidant, antimicrobial and anti-cancerous activity of methanol and chloroform leaf extract of Ficus auriculata (Moraceae) and Osyris wightiana (Santalaceae). Phytochemical study revealed the presence of carbohydrates, phenols, tannins, terpenoids, flavonoids and alkaloids in methanol and chloroform extracts of both plants. Glycosides were found only in the methanol extract and saponins were found absent in both plant extracts. The antioxidant activity of methanol and chloroform leaf extracts of both plants was evaluated by DPPH radical scavenging assay. In antioxidant activity assay, highest antioxidant potential was reported in O. wightiana leaf methanol extract where IC_{50} value lies in the conc. less than 20 µg/mL w/v. In antibacterial activity test, methanol and chloroform leaf extracts of Ficus auriculata and Osyris wightiana inhibited the growth of E. coli and S. typhimurium. Ficus auriculata leaf methanolic extract showed highest zone of inhibition against E. coli i.e. 18.33 ± 0.67 mm. Anti-cancerous activity of both plants was found to be insignificant at the conc. 100 µg/mL.
Keywords: *Ficus auriculata, Osyris wightiana, Phytochemical screening, Antioxidant activity, Antibacterial activity, Anti-cancerous activity*

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INTRODUCTION

Medicinal plants are the backbone of traditional medicine and have been used for the treatment of various infectious diseases. It is estimated that around 80 % of the world population depends on traditional medicine for primary health care and others depend on commercial drugs. Although we have current treatments such as antibiotics, bacteria are gaining resistance and thus reducing the effectiveness of commercial drugs [1]. Therefore, researchers are now focused towards the search of new antimicrobial substances from different parts of medicinal plants [2].

Ficus auriculata and *Osyris wightiana* are two important ethno-medicinal plants of Himachal Pradesh. *F. auriculata* Lour. (family Moraceae), also known as Roxburgh fig is an important medicinal plant of the state. Plants are found in temperate, tropical and sub-tropical regions from 1800-2600 m altitude above mean sea level [3]. Morphologically, the plant is dioecious and about 4-10 m tall. Every part of the plant contains abundant amount of white latex [4 and 5].

O. wightiana Wall. ex Wight, commonly known as Jangli Chai, is an evergreen shrub or small tree belonging to Santalaceae family. Its synonyms are *O. arborea* Wall., *O. arborea* f. *puberula* Hook., *O. divaricata* Pilger, *O. nepalensis* Griff. In India, plant is distributed in Himachal Pradesh, Uttarakhand, Arunachal Pradesh, Nagaland, Manipur, Orissa, Madhya Pradesh, Andhra Pradesh, Goa, Kerala, Tamil Nadu, Sikkim etc. Plant is approximately 1.2-9 m tall.

Ethno medicinally, *F. auriculata* has been reported for its medicinal properties and used in the treatment of diarrhea, dysentery, cuts, wounds, mumps, cholera, jaundice etc. [6]. *O. wightiana* is used in folk medicines and traditional Chinese medicines. *O. wightiana* leaves, stem and roots are used for the healing

of fractures [7]. Leaves are also reported for their emetic properties and antiviral activity [8]. Bark of the plant is used to make tea, which cures constipation and other stomach disorders. Besides these medicinal properties *F. auriculata* and *O. wightiana* plants also possess antimicrobial and antioxidant properties, reported in various studies [9, 10 and 11], but earlier studies didn't report antimicrobial and antioxidant properties of leaves of *F. auriculata* and *O. wightiana*. Therefore, present study is focused on the exploration of antioxidant and antimicrobial activity of methanol and chloroform leaf extracts of *F. auriculata* and *O. wightiana*. Keeping in view the importance of *F. auriculata* and *O. wightiana,* the present study is endeavored to study phytochemical, antioxidant, antibacterial, and anti-cancerous activity of methanol and chloroform leaf extracts of *F. auriculata* and *O. wightiana*.

MATERIAL AND METHODS

Sample collection and plant identification

The leaves of *F. auriculata* were collected from the trees grown in Shoolini university campus, Solan, H.P., situated at the elevation of 1352 m. The plants were identified and authenticated in the herbarium of School of Biological and Environmental Sciences, Faculty of Basic Sciences, Shoolini University, Solan. Leaves of *O. wightiana* were collected from trees grown in Tehsil Ghumarwin, District Bilaspur, H.P., situated at the elevation of 673 m (2,208 ft) above mean sea level. A herbarium was prepared from the plant and plant identification was done at Dr. Y. S. Parmar University of Horticulture and Forestry (UHF) Nauni, Solan, H.P. The herbarium sheet of *O. wightiana* was submitted to UHF-Herbarium with field book No.13556. The collected leaves of both plants were washed separately with double distilled water to remove soil and dust particles and were dried under shade for about two weeks and then grinded to form coarse powder and stored in airtight containers for further use. For antibacterial activity, bacterial strains of *Escherichia coli* and *Salmonella typhimurium* were obtained from microbiology research laboratory, Shoolini University, Solan.

Preparation of Leaf extract

Leaf extract was prepared by soaking 50 g of the dry, coarse powder of leaves in 100 mL of methanol and chloroform separately in two conical flasks. The flasks containing above mixture were kept on orbital shaker at 40ºC for 48 hrs. The process was repeated twice and the extracts were filtered and concentrated using hot water bath at 100°C. The dried or concentrated methanol and chloroform extracts were then stored in eppendorf tube for further use.

Preliminary phytochemical screening

The methanol and chloroform leaf extracts of both plant were studied for the presence of various phytochemical constituents like carbohydrates, alkaloids, flavonoids, glycosides, saponins, phenols, tannins, terpenoids using standard protocols [12 and 13].

Antioxidant Analysis

DPPH radical scavenging assay

The antioxidant activity of methanol and chloroform extracts of both plants was evaluated by DPPH radical scavenging assay [14]. Different conc. of leaf extracts (20, 40, 60, 80 and 100 µg/mL w/v) were prepared in methanol. 10 mL of freshly prepared DPPH solution (1mM) was mixed with 20 mL of different samples (20–100 µg/mL) and kept in dark for thirty minutes. Ascorbic acid (20–100 µg/mL w/v) was used as standard. After thirty minutes, the absorbance of the solutions was measured at 517 nm.

The DPPH radical scavenging activity was calculated using the following equation:

$$\%\ Free\ radical\ scavenging\ activity = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}}\ x\ 100$$

Where, $Abs_{control}$ represents absorbance of DPPH and Abs_{sample} represents absorbance of leaf extract and DPPH.

Antibacterial activity

Antibacterial activity of plant extracts was evaluated by standard disc diffusion method [15]. Methanol and chloroform crude extracts were dissolved in DMSO to make stock solutions of 80 mg/mL w/v.

Disc diffusion method

Bacterial inoculums were prepared using standard protocol CLSI M7-A7 [16] and turbidity of inoculums was compared with 0.5 McFarland standard containing 1-2 x 10^8 CFU/mL. Mueller Hinton Agar plates were prepared and 100 µl of bacterial inoculums was spread over the agar plates. The sterile discs containing 400, 550, 700 and 850 µg/mL w/v of leaf extract were placed on agar plates. Ampicillin was used as positive control and DMSO was used as negative control. The plates were incubated at 37ºC for 24 hrs. After incubation, zone of inhibition was measured using Hi antibiotic zone measurement scale.

Anti-cancerous activity

Anti-cancerous activity of the methanol and chloroform extracts of *F. auriculata* and *O. wightiana* was evaluated following Cytotoxicity assay- MTT [17 and 18]. Lung carcinoma A549 cells A549 (1 x 10^4) were seeded into the 96 well plate and cultured at 37°C in an atmosphere of 5% CO_2 to allow them to adhere overnight. Cells were then treated with 100 µg/mL w/v of different plant extracts (P1C, P1M, P2C, and P2M) and incubated at 37°C in an atmosphere of 5% CO_2 for 24 hrs. Vincristine sulfate and DMSO were used as positive and negative controls respectively. 10 µl MTT (5 mg/mL) was added into each well to generate Formosan and then cells were incubated in a humidified atmosphere with 5 % CO_2 at 37°C for 4 hrs. After removing the supernatant, 100µl DMSO was added to dissolve the purple crystals. The optical density of each well was measured at 595 nm by a micro plate reader. Each control and plant extract was assayed in duplicate for three times. The percentage of cell death was calculated by the following formula:

$$\%\ Cell\ death = \frac{OD_{control} - OD_{sample}}{OD_{control}}\ x\ 100$$

Where, $OD_{control}$ represents optical density for cells alone and OD_{sample} represents optical density for leaf extract treated cells.

Statistical analysis

All the experiments were done in triplicates, average and standard error were determined using GraphPad Prism 6.0.

RESULTS

Phytochemical screening of leaf extracts of both plants (*F. auriculata* and *O. wightiana*) revealed the presence of carbohydrates, phenols, tannins, terpenoids, flavonoids and alkaloids. Glycosides were present in methanol extract and absent in chloroform extract whereas saponins were absent in leaf extracts of both plants (Table 1).

Table 1: Phytochemical analysis of methanol and chloroform extracts of leaves of *Ficus auriculata* and *Osyris wightiana*

Test	*Ficus auriculata*		*Osyris wightiana*	
	ME	**CE**	**ME**	**CE**
Test for Carbohydrates				
Molish's test	+	+	+	+
Fehling's test	+	+	+	-
Benedict's test	+	+	+	+
Test for Tannins and Phenols				
Ferric chloride test	+	+	+	-
Lead acetate test	+	+	+	+
Test for Glycosides				
Keller-Killiani test	+	-	+	-
Test for Saponins				
Foam test	-	-	-	-
Test for Triterpinoids				
Salkowaski test	+	+	+	+
Test for Flavonoids				
Alkaline reagent test	+	+	+	+
Test for Alkaloids				
Mayer test	+	+	+	-
Wagner's test	+	+	+	-
Dragendroff's test	+	+	+	+

+ = Positive, - =Negative, ME = Methanol extract, CE = Chloroform extract

Antioxidant activity of leaf extracts of *F. auriculata* and *O. wightiana*

The antioxidant analysis of leaf extracts was assayed by DPPH radical scavenging assay.

2,2-diphenyl-1-picrylhydrazyl (*DPPH) radical scavenging assay*

In DPPH radical scavenging assay, the percentage scavenging of DPPH radical was increased with increase in the conc. (20-100 µg/mL w/v) of leaf extracts of both plants (Fig. 1). IC_{50} value of *F. auricluata*

methanol extract lies in the conc. range of 40-60 µg/mL w/v whereas the IC_{50} value of chloroform extract lies in the conc. range of 80-100 µg/mL w/v. IC_{50} value of *O. wightiana* leaf methanol extract lies in the conc. less than 20 µg/mL whereas the IC_{50} value of chloroform extract lies in the conc. range of 80-100 µg/mL (Fig. 1).

Antibacterial activity of leaf extracts of *F. auriculata* and *O. wightiana*

Different conc. of leaf extracts were used for antimicrobial activity assay. Both the tested bacterial pathogens were found susceptible to the leaf extracts of *F. auriculata* and *O. wightiana* (Table 2, 3). *Ficus auriculata* leaf methanolic extract showed highest zone of inhibition against *E. coli* i.e. 18.33 ± 0.67 mm while leaf chloroform extract showed highest zone of inhibition against *S. typhimurium* i.e. 17.67 ± 0.33 mm at conc. of 850 µg/mL w/v (Table 2). *Osyris wightiana* leaf methanolic and chloroform extracts showed highest zone of inhibition against *Salmonella typhimurium* i.e. 16.66 ± 0.33 mm and 17.33 ± 0.33 mm respectively at conc. of 850 µg/mL w/v (Table 3).

Anti-cancerous activity of leaf extracts of *F. auriculata* and *O. wightiana*

To evaluate anti-cancerous activity, A549 cancer cells were treated with leaf extracts of *O. wightiana* and *F. auriculata* (100 µg/mL w/v). The cytotoxicity assay demonstrated that there was no significant cancer cell killing with P1C, P1M, P2C and P2M (Fig. 2).

DISCUSSION

In the present study, phytochemical, antioxidant, antibacterial and anti-cancerous activity of leaf extracts of *F. auriculata* and *O. wightiana* was evaluated. In phytochemical testing, *F. auriculata* and *O. wightiana* leaf extracts were found rich in alkaloids, phenols, flavonoids, tannins and terpenoids (Table 1). Leaf extracts of both plants exhibited antioxidant activity. In antioxidant analysis, the percentage scavenging of DPPH increased with increase in the conc. of leaf extracts. *O. wightiana* leaf methanol extract was found rich in antioxidants when compared with other other tested leaf extracts. IC_{50} value of *O. wightiana* leaf methanol extract lies in the conc. < 20 µg/mL (Fig. 1). The results of the present study were compared with the existing literature. The antioxidant activity of plants is associated with the presence of phenolic compounds and flavonoids [19]. In this study, both methanol and chloroform leaf extracts showed positive tests for presence of phenols and flavonoids. The antimicrobial activity of methanol and chloroform leaf extracts of *F. auriculata* and *O. wightiana* was tested *in vitro* against two bacterial pathogens (*E. coli* and *S. typhimurium*). Results of antibacterial study showed that both plants are effective against *E. coli* and *S. typhimurium* (Table 2, 3). The pathogenic strains of *E. coli* cause diarrhea, abdominal pain and fever while *S. typhimurium* cause gastroenteritis in humans. There are many reports on antioxidant, antimicrobial and anticancer activity of plants and the antimicrobial activity of plants are associated with the presence of alkaloids [20, 21 and 22]. There are reports that flavonoids possess multiple biological property including antimicrobial, cytotoxicity and anti-inflammatory [23]. Additionally, tannins also play important role in antimicrobial activity [24]. Flavonoids and tannins were found present in methanol and chloroform extracts of both plants. So, in the present study, antibacterial activity of leaf extracts was due to the presence of tannins and flavonoids. The anti-cancerous activity of leaf extracts of both plants was evaluated using A549 cancer cells and results showed that leaf extracts were not effective against A549 cancer cells at 100 µg/mL w/v of leaf extract (Fig. 2).

Table 2: *In vitro* antibacterial activity of methanol and chloroform leaf extracts of *Ficus auriculata* against bacterial pathogens

Microbes	IZD mean (mm) ± S.D.								
	Methanol extract Conc. (µg/mL)				Chloroform extract Conc. (µg/mL)				Ampicillin Conc. (µg/mL)
	400	**550**	**700**	**850**	**400**	**550**	**700**	**850**	**10**
Escherichia coli	12.33± 0.89	15.67± 1.33	17.67± 0.33	18.33± 0.67	12± 0.57	12.66± 0.66	13.33± 0.33	14.33± 0.88	10±0.33
Salmonella typhimurium	11.33± 0.33	13.67± 0.67	16.00± 0.00	17.67± 0.33	13.33± 0.66	14.33± 0.66	14.66± 0.88	16± 1.15	11±0.33

IZD = Inhibition zone diameter, S.D. = Standard Deviation

Table 3: *In vitro* antibacterial activity of methanol and chloroform leaf extracts of *Osyris auriculata* against bacterial pathogens

Microbes	IZD mean (mm) ± S.D. Methanol extract Conc. (µg/mL)				Chloroform extract Conc. (µg/mL)				Ampicillin Conc. (µg/mL)
	400	**550**	**700**	**850**	**400**	**550**	**700**	**850**	**10**
Escherichia coli	12± 0.57	12.66± 0.66	13± 0.58	13.33± 0.33	12± 0.57	13.33± 0.88	13.66± 0.66	14.66± 0.66	13.57± 0.04
Salmonella typhimurium	14± 1.15	14.33± 0.88	14.66±0.33	16.66± 0.33	13.33± 0.33	14.33± 0.66	15.33± 0.33	17.33± 0.33	10.23± 0.52

IZD = Inhibition zone diameter, S.D. = Standard Deviation

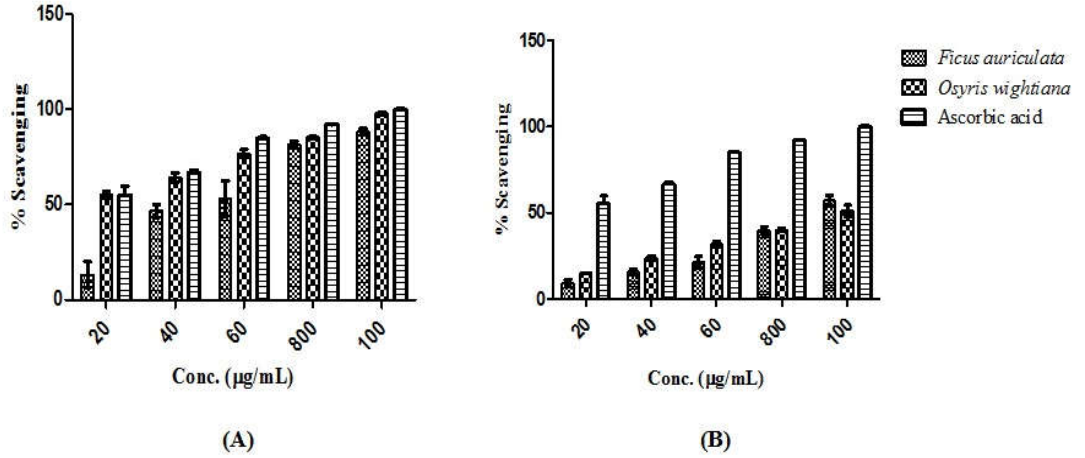

Fig. 1: DPPH radical scavenging activity of methanol extracts (A) and chloroform extracts (B) of leaves of *Ficus auriculata* and *Osyris wightiana* at different concentration (20-100 µg/mL w/v). Ascorbic acid (20-100 µg/mL w/v) was used as standard.

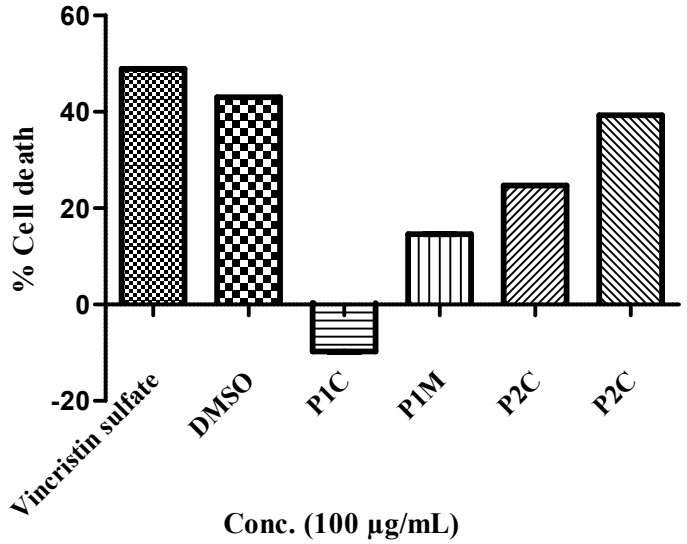

Fig. 2: Anti-cancerous activity of *Ficus auriculata* and *Osyris wightiana* leaf extracts. Vincristin sulphate was used as positive control and DMSO was used as negative control
P1C= *F. auriculata* chloroform leaf extract P2C= *O. wightiana* chloroform leaf extract P1M = *F. auriculata* methanol leaf extract P2M= *O. wightiana* methanol leaf extract

CONCLUSION

In present study methanol and chloroform leaf extracts of *Ficus auriculata* and *Osyris wightiana* successfully inhibited the growth of *E. coli* and *S. typhimurium.* These plants were found rich in phytochemicals and showed strong antioxidant potential by scavenging of DPPH radical. Anti-cancerous activity of both plants was found to be insignificant at 100 µg/mL. However, in future, the anti-cancerous activity of these plants at higher concentration and broad spectrum antimicrobial activity needs to be explored to use these plants as a drug developing candidate.

CONFLICT OF INTEREST

None.

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