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Antibacterial, antifungal, antioxidant and phytochemical study on the leaves extract of *Grewia optiva*

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ABSTRACT

The World Health Organization estimates that 80% of the world's population presently uses herbal medicine for some aspect of primary health care. The use of, and search for, drugs and dietary supplements derived from plants have accelerated in recent years.The genus *Grewia* is mainly associated with triterpenoids and alkaloids. Various parts of the plants has been used to treat many diseases. In continuation of this, the work is being pursued on the phytochemical, antimicrobial and antioxidant investigation on the leaves of *Grewia optiva*.Successive extraction of the crushed leaves of the plants has been done with various solvents viz. petroleum ether, benzene, chloroform, acetone, methanol and water respectively. The different extracts of the leaves of *Grewia optiva* were tested quantitatively. Some new phytoconstituents: Fats and fixed oils were found in Petroleum ether, benzene, chloroform, acetone, methanol and water extract; Protein and amino acids were found in acetone, methanol and water extract and Saponins was found in methanol and water extract.These extracts were further tested for their antimicrobial activity against the bacterial strains of *Escherichia coli, Klebsiella pneumonaie., Pseudomonas aeruginosa, Proteus mirabilis* and against the fungal strains of *Aspergillus niger, Microsporus gypseum, Rhizopus stolonifer, Aspergillus japanicus* and *Penicillum chrysogenum* .The results have shown that the all extracts showed very good activity against the bacterial strains of *Pseudomonas aeruginosa* and methanol extract can act as standard drug against this strain as it showed more inhibition zone than the standard drug doxycycline. The extract showed very weak activity against all the tested fungal strains.They showed no remarkable antioxidant activity in methanol and acetone extract.

Key words: *Grewia optiva*, Antibacterial activity, Antifungal activity, Antioxidant activity.

INTRODUCTION

The use of drugs is just as old as surgery. Plants also play a role in modern chemistry beyond lending their molecular structure to synthesized drugs.The number of higher plant species on this planet is estimated at 250,000,[1] with a lower level at 215,000 [2, 3] and an upper level as high as 500,000.[4, 5] Of these, only about 6% have been screened for biological activity and reported 15% have been evaluated phytochemically.[6] Fossil records date human use of plants as medicines at least to the some 60,000 years ago.[7] In fact, according to the World Health Organization, approximately 25% of modern drugs used in the United States have been derived from plants.[8]

The extracts from the plant of *Grewia* are known to have medicinal properties.[9] The various parts of *Grewia* are used to treat skin diseases, hypertension, cancer, malaria, tranquilizer etc. [10-16] In continuation of our efforts in search of potential antimicrobial and antioxidant agent with no side effect, we have taken a new species of *Grewia* i.e. *Grewia optiva* and screened their leaves for antimicrobial and antioxidant activity.

2 MATERIALS AND METHODS

Experimental work was carried out on the leaves of *Grewia optiva* under the three heads: Phytochemical investigation of extracts, Antimicrobial activity of leaves extracts and antioxidant activity of promising extracts.

2.1 Phytochemical investigation of extracts

2.1a) Collection of plant material

The plant leaves was collected from outside the Institute campus Dolphin (PG)Institute of Biomedical and Natural Sciences, (DIBNS) Manduwala, Dehradun during the month of Oct. to Dec. Collected plant material was authenticated by Dr. Rakesh Kumar Head, Department of Biosciences, DIBNS. The collected plant material was washed with water to remove mud and other undesirable material and dried under shade.

2.1b) Preparation of extracts

The air-dried leaves (50gm) of *Grewia optiva* were crushed and powdered separately. The powdered leaves were extracted with different solvents of increasing polarity viz. petroleum ether, benzene, chloroform, acetone, methanol and water.

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2.1c) Qualitative phytochemical test

The different extract of leaves of *Grewia optiva* were tested for various components by their specific tests viz. Mayer's test, Dragendroff's test, Wagner's test for alkaloids; Raymond's test, Legal's test, Bromine water test for glycosides; Gelatin test, Ferric chloride test, Vanillin hydrochloride test for tannins & phenolic compounds; Shinoda test (Magnesium hydrochloride reduction test), Zinc hydrochloric reduction test, Alkaline test for flavonoids; Million test, Ninhydrin test, Xanthoproteic test for proteins and amino acids; Salkowski test, Sulfur powder test for sterols and triterpenoids; Molisch's test, Benedict's test, Barfoed's test, Bromine water test for carbohydrates; Spot test, Saponification test for fats and fixed oils and Foam test for saponins.

2.2) Antimicrobial activity of extracts

The antimicrobial activity of the leaves extracts of *Grewia optiva* were carried out. The leaves extracts were screened for antibacterial and antifungal activities.

2.2a) Antibacterial activity of leaves extracts

The bacterial cultures used in the study were *Escherichia coli, Klebsiella pneumonaie, Pseudomonas aeruginosa, Proteus mirabilis.* These bacterias were provided by Department of Microbiology, Dolphin Institute of Biomedical and Natural Sciences, Manduwala, Dehradun and checked for purity by convention biochemical methods. These bacterial cultures were maintained on nutrient agar slants at first being incubated at 37ºC for about 18-24 hours and then stored at 4ºC as stock cultures for further antibacterial activity. Fresh culture were obtained by transferring a loop full of culture into nutrient broth and then incubated at 37ºC overnight. To test antibacterial activity, the well diffusion method was used.

Culture media preparation

The microbiological media prepared as standard instruction provided by the HI-MEDIA Laboratories Pvt. Ltd., Mumbai. The medium used for antibacterial activity were Mueller-Hinton Agar (MHA) and Nutrient Broth (NB). They were prepared and sterilized at 121ºC at 15 psi for 15-30 minutes in autoclave.

Plate preparation

25ml of pre autoclaved Mueller-Hinton agar (MHA) was poured into 90 mm diameter pre sterilized petri plates. These petri plates were allowed to solidify at room temperature.

Well diffusion method

After the plates solidified the freshly prepared microbial broth culture suspension (about 0.1 ml) was spreaded over the Mueller-Hinton agar (MHA) media

using L shaped sterilized glass spreader separately under aseptic condition using laminar air flow. Then wells were made in each plate with the help of borer of 8 mm diameter. In these well, about 0.1 ml of each leaves extracts individually was loaded. This method depends upon the diffusion of leaves extracts from hole through the solidified agar layer of petri dish to such an extent that the growth of added micro organism is prevented entirely in a circular area or zone around the hole containing leaf extract. Petri plates were incubated for 24 hrs at 37ºC in the incubator. After incubation, the diameter of clear zone of inhibition produced around the well or holes were measured in mm and compared with standard drug.

2.2b) Antifungal activity of leaves extracts

In this study, the antifungal activity was studied against the microorganism viz. *Aspergillus niger*, *Rhizopus stolonifer*, *Microsporum gypseum, Aspergillus japanicus and Penicillium chrysogenum.* These cultures were obtained from the standard cultures maintained in the Microbiology Department of DIBNS, Dehradun. These cultures were maintained on Sabouraud Dextrose Agar (SDA) at first being incubated at 25ºC for about 72-96 hours and then stored at 4ºC as stock cultures for further antifungal activity. Fresh cultures were obtained by transferring a loop full of cultures into sabouraud dextrose broth and then incubated at 25ºC for 72 hrs. To test antifungal activity, the well diffusion method was used. Here culture media preparation in sabouraud dextrose agar (SDA) and incubation period is 72 hours at 25ºC rest the method is same as that of antibacterial activity.

2.3 Antioxidant activity

Free radical scavenging activity by 2, 2-diphenyl-1-picrylhydrazyl (DPPH)

The free radical scavenging activity of concentrated methanolic leaves extract was calculated with the help of DPPH by the method of Blosis.[17] 0.1mM solution of DPPH in methanol was prepared and 1ml of this solution was added to 3ml of various quantities of concentrated methanolic leaves extract (50, 100,150, 200, 250µg) and in the reference compound. After 30min absorbance was measured at 517 nm. Butylated hydroxyl anisole (BHA) is used as reference compound. Similar procedure was followed for the acetone extract of leaves. Percentage inhibition was calculated by comparing the absorbance of control and sample. Lower absorbance of reaction mixture indicates the higher scavenging activity.

3. RESULTS AND DISCUSSION

The various extracts of the leaves of *Grewia optiva* were prepared and studied for phytochemical investigation and antimicrobial activity.

3.1 Phytochemical investigation of extracts

In petroleum ether extract yield was highest while in chloroform we got the least yield.The extracts of the leaves of *Grewia optiva* undergoes various qualitative chemical test. They showed their presence and absence in the different solvent systems which is summerized in **Table-1**. From the **Table-1** we can find out that methanol extract was the richest extract for phytoconstituents. Except tannins of phenolic compounds,carbohydrates and flavonoids it contains all tested phytoconstituents viz. Alkaloids , glycosides, proteins and amino acids triterpinoids of sterol , fats and fixed oil and saponins. Next is water extract that contain protein and amino acids, fats and fixed oil and saponins. Again acetone extract contain protein and amino acid, triterpenoids of sterol and fats and fixed oil. Petroleum ether, benzene and chloroform extracts contain only triterpenoids of sterol and fats and fixed oil

Table 1 Qualitative chemical test of various extracts of the leaves of *Grewia optiva*

S.no.	Phyto-constituents	Petroleum ether	Benzene	Chloroform	Acetone	Methanol	Water
1.	Alkaloids	-	-	-	-	+	-
2.	Glycosides	-	-	-	-	+	-
3.	Tannins and Phenolic Compounds	-	-	-	-	-	-
4.	Flavonoids	-	-	-	-	-	-
5.	Protein and amino acids	-	-	-	+	+	+
6.	Triterpenoids And sterols	+	+	+	+	+	-
7.	Carbohydrates	-	-	-	-	-	-
8.	Fats and fixed oils	+	+	+	+	+	+
9.	Saponins	-	-	-	-	+	+

Key

1. *- = ABSENT*

2. *+ =PRESENT*

3.2 Antimicrobial activity of extracts

The leaves extracts (Petroleum ether, benzene, chloroform, acetone, methanol and water) were screened for antibacterial and antifungal activities.

3.2a) Antibacterial activity

The antibacterial activity of different extracts of *Grewia optiva* and standard drugs Doxycycline were tested for different strains of bacteria and zone of inhibition was recorded in millimeter **(Table-2)**. In table 2 antibacterial activities of different extracts against tested microorganism are shown. Chloroform extracts showed antibacterial activity against *Pseudomonas aeruginosa* and *Escherichia coli*. Methanol, Acetone, Petroleum ether and benzene extracts showed antibacterial activity only against *Pseudomonas aeruginosa*. Lastly water extract showed antibacterial activity only against*Klebsiella pneumoniae*. Except water extract all other extracts showed antibacterial activity against *Pseudomonas aeruginosa* in which methanol extract showed the highest activity. Methanol extract not only showed highest activity among all the extracts but it also showed highest antibacterial activity against*Pseudomonas aeruginosa* in comparison to standard drug Doxycycline.

Table-2 Antibacterial activity of different extracts of *Grewia optiva* and standard drug Doxycycline

S.No.	Test organism	Inhibition zone in mm Pet. ether	Benz-ene	Chloro-form	Acet-one	Meth-anol	Water	StandardDrug Doxycycline
1.	*E.coli*	-	-	18	-	-	-	33
2.	*K.pneumonie*	-	-	-	-	-	12	21
3.	*P.aeruginosa*	**20**	**18**	**20**	**25**	**32**	-	**26**
4.	*P. mirabilis*	-	-	-	-	-	-	22

3.2b) Antifungal activity

Antifungal activity of different extracts of *Grewia optiva* and standard drug Fluconazole were tested for different strains of fungus and recorded the zone of inhibition in millimeters (**Table-3).** Among all the extracts water extract shows activity against*A. niger* and *A. japanics*. Petroleum ether extract shows activity against *Rh. Stolonifer*. Chloroform and Methanol extract shows activity against *A. japanics*.

Table-3 Antifungal activity of different extracts of *Grewiaoptiva* and standard drug Fluconazole

S.No	Test organism	Inhibition zone in mm Pet. ether	Ben-zene	Chlor-oform	Acet-one	Metha-nol	Water	Standard Drug Fluconazo-le
1.	*A.niger*	-	-	-	-	-	13	23
2.	*R.stolonifer*	12	-	-	-	-	-	25
3.	*M.gypseum*	-	-		-	-	-	30
4.	*A. japanicus*	-	-	11	-	17	17	31
5.	*P.chrysogen-um*	-	-	-	-	-	-	28

3.3 Antioxidant activity

Free radical scavenging activity by 2, 2-diphenyl-1-picrylhydrazyl (DPPH)

Free radical scavenging activity by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) is

Table-4 Antioxidant activity

Absorbance table of methanolic extract of *Grewia optiva* and BHA at 517nm

Conc. mcg/ml	Methanol extract	BHA
50	0.07	0.065
100	0.085	0.046
150	0.092	0.039
200	0.092	0.032
250	0.091	0.028

Absorbance table of acetone extract of *Grewia optiva* and BHA at 517nm

Conc. mcg/ml	Acetone extract
50	0.025
100	0.047
150	0.057
200	0.066
250	0.093

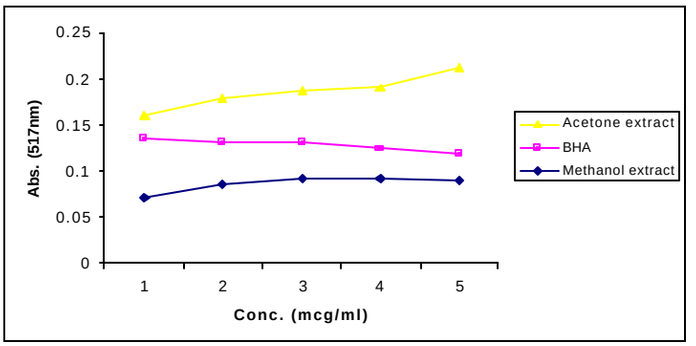

Fig.-1 Plot for free redial scavenging activity by DPPH

usually used as a reagent to evaluate free radical scavenging activity of antioxidants. DPPH is a free radical and accept an electron of hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radical is determined by the decrease in absorbance at 517nm induced by the antioxidants. BHA was used as standard reagent **(Table-4)**. Result showed that the absorbance of concentrated metanolic leaves extract and acetone extract of *Grewia optiva* increases with increase in the concentration. Hence both of the extract does not possess antioxidant activity. Where as in standard, BHA showed decrease in the absorbance with increase in concentration **(Figure-1)**.

4. CONCLUSION

The methanol extract of leaves of the *Grewia optiva* have maximum test phytoconstitutes and methanol extract can act as standard drug against bacterial strain *Pseudomonas aeruginosa* as it showed more inhibition zone than the standard drug Doxycycline. In fungal strains none of the leaves extract showed more inhibition zone than standard drug Fluconazole. In the study of antioxidant activity *Grewia optiva* leaves shows there is an increase in the absorbance with increase in the concentration of methanol and acetone extract of leaves of *Grewia optiva*, so methanol and acetone extract of leaves of *Grewia optiva* does not show antioxidant activity.

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