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Phytochemical investigation, antibacterial activity and antioxidant activity of the endangered tree *Commiphora wightii* (Arn.) Bhandari

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Abstract

Commiphora wightii is an arid region plant, highly valued for its medicinally important guggul gum-resin as a source of guggulsterone. It is listed in IUCN's Red Data List of threatened plants and now it is becoming endangered. Gum resin it has been used in the Ayurveda science time immemorial for the treatment of variety of disorders such as inflammation, rheumatism, obesity and disorders of lipids metabolism. The present paper deals with phytochemical studies in antibacterial activity and antioxidant activity of *C. wightii*. Qualitative analysis of *C. wightii* shows the presence of bioactive compounds such as Alkaloids Flavonoids, Phenols, Tannins, Steroids and Terpenoids were present in *C. wightii* except, Saponins and Carbohydrates. Study confirms the antibacterial activity of gum extract of *C. wightii* the extract found effective bacterial strain, the activity of gum extract antibacterial activity higher than in gram negative bacteria, gum had a broad spectrum antibacterial activity of compare to in gram positive bacteria is a plant with a variety of ethnic medicinal uses. Antioxidant activity by inhibiting DPPH free radicals which indicates the gum extract is very much of *C. wightii* can be used as an accessible source of natural antioxidant agent. This is valuable information for preparation of drugs in pharmaceutical industry and stresses the need for more intensive research since they play a great role in healthcare.

Keywords: *Commiphora wightii*, guggul gum, phytochemical analysis, qualitative analysis, antibacterial activity, antioxidant activity

Introduction

Commiphora wightii (Arn.) Bhandari a medicinally important endangered plant that belongs to family Burseraceae, commonly known as 'Indian bdellium' in English, as Mahisaksha, Guggulu, Amish, Palanksha and Pur in Sanskrit and as Guggul in most Indian languages [1]. The distribution of *C. wightii* is restricted to the Aravalli hills (India) like Gujarat and Rajasthan, It is much branched, dioecious, up to 4 m tall with brown coloured, spine scented knotty, crooked and spirally ascending branches ending in sharp spines [2]. Bark shiny, ash to yellowish white coming off in rough flakes exposing the greenish under bark, which also peels off in thin papery rolls. Leaves small, sessile, 1-2 leaflets, highly aromatic, leathery, and shinning green on top greyish below with irregularly toothed edges. Flowers small, unisexual, sessile, brownish red, occurring singly or in groups of 2-4, 7-9 lobed disc and an oblong-ovoid ovary; stamen 9-10. Fruit an ovoid green berry like drupe, reddish, 7-8 mm in diameter. Seed generally contain an under developed embryo. The generic name is derived from Greek 'phora' and 'kommis' meaning gum bearer. This is a threatened and vulnerable species due to its over-exploitation [3]. It came in vulnerable list in the 1997 IUCN Red List as vulnerable (VU) and in the 2004 IUCN Red List as data deficiency (DD) (IUCN, 2004). The Government of India has recently banned the export of the gum (IUCN, 2004), due to its high market price in international trade [4].

Commiphora wightii, a highly valuable medicinal plant yields an oleo-gum resin important in Ayurvedic medicines [5]. Medicinal importance of *Commiphora wightii* is known since ancient times as it is mentioned by Sushruta 3000 years ago as a valuable drug in Ayurveda. The plant contains essential oil, mainly consisting of myrcene, dimyrcene and polymyrcene, E-guggulsterone, Z-guggulsterone, guggulsterone-I, II. These isolates have been found useful in curing many diseases like rheumatism, arthritis, hyperlipidemia, obesity, inflammation, anti-bacterial, antimicrobial, anti-oxidant, anti-arthritic, anti-malarial, muscle relaxing, larvicidal and Guggulsterone- E and Z the active constitute of resin are responsible for lipid lowering properties in human blood and these flavonoids can be described as pharmacotherapeutics as they can help in the treatment of diseases such as hypercholesterolemia, hypertension, obesity and diabetes [6-8].

In India the gum resin has also been used for treating various types of Arthritis. Ayurvedic physicians extensively used guggul gum for treating arthritis and related conditions for centuries. Bhils take the powder of bark orally with water to cure cough and cold. They also inhale the fumes of gum resin to cure fever, bronchitis, nasal congestion, laryngitis and phthisis. Gracias tribals dissolve the gum resin in warm water and use for gargling against pyorrhea, tonsillitis and pharyngitis. Tribals of Barmer give the gum orally to the children suffering from speech defects. Saharia tribals apply the paste of gum resin on cuts and injuries for early healing. The Kalbelia nomadic tribals take the fresh decoction of plant orally to cure asthma. The twigs are used as toothbrush and due to its highly medicinal importance, it is becoming

endangered and there is a need for its conservation. The current study focused on phytochemical investigation, antibacterial activity and antioxidant activity of *C. wightii* (gum-resin) plant.

Materials and methods

Plant and gum of *Commiphora wightii* were collected from Herbal garden, Prof. Jayashankar Telangana State Agricultural University, Hyderabad and planted in the Botanical Garden at Department of Botany, Osmania University, Hyderabad. These plants gum were subjected to Phytochemical analysis for the presence of several medicinally important compounds, antibacterial and antioxidant activity gum extract (Fig-1).



Fig:- 1. Gum of *Commiphora wightii*

Preparation of extracts

Plant samples gum were washed with distilled water and air-dried at room temperature for 7-10 days, then oven-dried at 40 °C to remove the residual moisture. The dried plant parts were pulverized and stored in air-tight containers at 4 °C for future use. 50 g of powdered samples of gum were extracted with methanol by soxhlation method at 60 to 80 °C. The three filtrates were separately concentrated in water bath at 40 °C and evaporated under reduced pressure.

Phytochemical analysis

The extracts obtained from the powdered gum of *Commiphora wightii* were subjected to phytochemical tests to determine the presence of active secondary metabolites using standard procedures [9]. This extract was filtered through a fine mesh into a test tube. This crude extract was used for the qualitative tests given below and the tests were carried out in triplicate.

Qualitative analysis

It comprised of tests for the presence of Alkaloids, Tannins, Glycosides, Carbohydrates, Steroids, Saponins, Flavonoids and Phenols.

Test for Alkaloids

About 0.5 gm of methanol gum extract was taken in a test tube and was diluted and homogenized with 10 ml distilled water, dissolved in 20 ml dilute HCl solution and clarified by filtration. The filtrate was tested with Dragendorff's and Mayer's reagent. The treated solution was observed for precipitation of white or creamy colour.

Test for Tannins

Five gm of the ground gum powder was extracted with 10 ml ammonical chloroform and 5 ml chloroform. The mixture was filtered and the filtrate was shaken with 10 drops of 0.5M sulphuric acid. Creamish white precipitate was observed for the presence of tannins.

Test for Glycosides

About 0.5 gm of methanol gum extract was taken in a test tube and 1 ml glacial acetic acid containing traces of ferric chloride was added to it. To this solution, 1 ml concentrated sulphuric acid was added and observed for the formation of reddish brown colour at the junction of the two layers and the upper layer turned bluish green in the presence of glycosides.

Test for Resins

About 0.5 gm of methanol gum extract was taken in a test tube and 5 ml of distilled water was added to it and observed for turbidity which indicates the presence of resins.

Test for Steroids

About 0.5 gm of methanol gum extract was taken in a test tube and 2 ml of acetic anhydride was added to it and 2 ml of sulphuric acid was added by the sides of the test tube and observed for the colour change to violet or blue green.

Test for Saponins

About 0.5 gm of methanol gum extract was taken in a test tube and 5 ml distilled water was added to it. The solution was shaken vigorously and observed for persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for Flavonoids

About 0.5 gm of gum extract was introduced into 10 ml of ethyl acetate in a test tube and heated in boiling water for 1 min. The mixture was then filtered. About 4 ml of the filtrate was shaken with 1 ml 1% aluminium chloride solution and incubated for 10 min. Formation of yellow colour in the presence of 1 ml dilute ammonia solution indicated the presence of flavonoids.

Test for Phenols

About 0.5 gm of gum extract was taken in a test tube, mixed with 100ml distilled water and heated gently. To this, 2 ml of ferric chloride solution was added and observed for the formation of green or blue colour.

Test for carbohydrate

Benedict's test: gum extracts were mixed with 2ml of Benedict's reagent and boil, and observed for the formation of reddish brown precipitate, which indicates the presence of the carbohydrates.

Antibacterial Activity

The disc diffusion method was used to evaluate the antibacterial activity of the synthesized compounds against four bacterial strains viz; *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *S. aureus* *Bacillus subtilis* and *Bacillus thuringiensis*. Each organism was cultured in nutrient broth at 37 °C for 24 h. Then 1% broth culture containing approximately 106 colony forming units (CFU/mL) of test strain was added to nutrient agar medium at 45 °C and poured into sterile petri plates. The medium was allowed to solidify. 5 µL of the test compound (40 mg/mL in DMSO) was poured on 4 mm sterile paper discs and placed on nutrient agar plates. In each plate standard antibacterial drug (ampicillin) and metal complexes were added. The plates were incubated at 37 °C for 24 h and the antibacterial activity was determined by measuring the diameter of zones showing complete inhibition (mm) using standard procedures reported in medicinal plants [10].

Table I: Phytochemical (qualitative) analysis of the gum extracts of methanol *Commiphora wightii*.

S. NO	Test for Phytochemicals	Test results
		Gum extract
1	Alkaloids	+ve
2	Flavonoids	+ve
3	Phenols	+ve
4	Steroids	+ve
5	Tannins	-ve
6	Terpenoids	+ve
7	Saponins	-ve
8	Carbohydrates	-ve

The antibacterial screening of the gum extract of *C. wightii* were performed against gram negative bacteria (*E.coli*, *K. pneumoniae* and *P. aeruginosa*) and gram positive (*S. aureus*, *Bacillus subtilis* and *Bacillus thuringiensis*) by the disk diffusion method. The activities of the compounds were compared with standard Ampicillin for antibacterial activity. The antibacterial properties of the imine base and its solvent extract evaluated and presenting in Fig-1 and Table-2, indicated that the compounds are active in exhibiting antibacterial role like gum 0.4, 0.4 and 0.6 Minimum inhibition zone in gram negative bacteria and leaf 0.4, 0.3 and 0.4 Minimum inhibition zones in gram positive bacteria.

Radical Scavenging Activity

The percentage of free radical scavenging activity is shown in Fig-2. This assay is based on decrease in absorbance value of DPPH at 517 nm on addition of complex. The experiment involves diluting the working solution of the plant gum extracts and the ascorbic acid standard (700, 600, 500, 400, 300 and 200 µg/µL⁻¹) in methanol. DPPH concentration was kept constant (2 mL, 0.004%). To this varying concentration of plant extracts and standard were added. The mixture was shaken vigorously and kept in dark for 30 min at room temperature. Then the absorbance was measured at 517 nm in a spectrophotometer. The whole experiment was carried out using spectroscopic grade methanol solvent at 298 K. The radical scavenging activity has been measured by using the following Eq. 1;

$$\text{Suppression ratio (\%)} = [(A_0 - A_i) / A_0] \times 100\% \quad (1)$$

Where A_i =the absorbance in the presence of the ligand or its complexes, A_0 =the absorbance in the absence of the ligand or its plant extracts.

Results and Discussion

Qualitative analysis of gum extract *C. wightii* was carried out for Alkaloids, Flavonoids, Phenols, Saponins, Carbohydrates, Proteins, Steroids, Tannins, and Terpenoids. All of the phytochemicals like Alkaloids Flavonoids, Phenols, Tannins, Steroids and Terpenoids were present in *C. wightii* except, Saponins and Carbohydrates (Table-1). The present study also reports Similar findings were reported in medicinal plants like [11-14]. Alkaloids and Saponins are known to be effective for the treatment of syphilis and other venereal diseases, had earlier reported that Saponins have antibiotic properties and so help the body to fight infections and microbial invasion. Also, it is used as a mild detergent and in intracellular histochemistry staining to allow antibody access to intracellular proteins; these proteins were also reported in hyperglycemia, antioxidant, anticancer, anti-inflammatory and weight loss and have anti-fungal properties in *C. wightii*.

Study confirms the antibacterial activity of gum extract of *C. wightii* the extract found effective bacterial strain, the activity of gum extract antibacterial activity higher than in gram negative bacteria, whereas more when compare to in gram positive bacteria. Gum extract were obtained from different samples ranged from 15 to 17% (w/w) i.e., average recovery was comparatively less than reported earlier probably due to variation in quality of different gum samples, evident from the study and earlier reports, However reported in the present study which agrees with the findings of [15-17, 10].

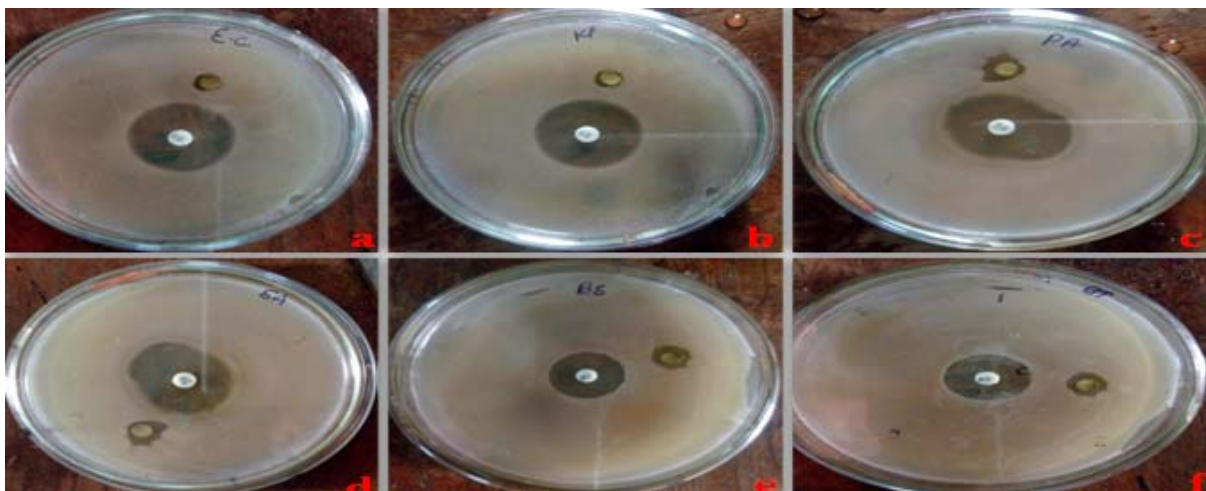


Fig 1: Antibacterial activity of gum extract of *C. wightii* (a) *E. coli*, (b) *P. aeruginosa*, (c) *K. pneumoniae*, (Gram Negative) and (d) *S. aureus*, (e) *B. subtilis*, (f) *B. thuringiensis* (Gram Positive) ampicillin as positive control.

Table 2: Minimum inhibition zone (mm) complexes ($\mu\text{g/ml}$) gum extract of *C. wightii*.

Bacterial inhibition zone (mm) Gram (+)			Bacterial inhibition zone (mm) Gram (-)		
<i>E. coli</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>	<i>B. subtilis</i>	<i>B. thuringiensis</i>
0.4	0.4	0.6	0.4	0.3	0.4

Antioxidant Properties

The model of scavenging the stable DPPH radical is a widely used technique to screen antioxidant properties by spectrophotometer in a very short time period. When the reaction between antioxidant molecule and DPPH radical occurs, it results in decrease in absorbance at 517 nm. This is because the radical is scavenged by antioxidants through donation of hydrogen to form the reduced form (DPPH-H), and this property is also visually noticeable as the color changes from purple to yellow. The more rapidly the absorbance decreases, the more potent is the antioxidant compound. In the present study the antioxidant activity of Gum extract of *C. wightii* was evaluated by scavenging stable DPPH radical (Fig:-2). The DPPH radical scavenging activities were found to be 62.56% for ascorbic acid, 16.84% for Gum extract, at concentration of the 200 $\mu\text{g}/\mu\text{L}^{-1}$, 74.45%

29.80% at 300 $\mu\text{g}/\mu\text{L}^{-1}$, 86.98% 39.11 at 400 $\mu\text{g}/\mu\text{L}^{-1}$, 89.98% 57.08% at 500 $\mu\text{g}/\mu\text{L}^{-1}$, 90.99% 64.05% at 600 $\mu\text{g}/\mu\text{L}^{-1}$, Ascorbic acid exhibited higher DPPH scavenging activity than the compound at all concentrations. At the concentration of 700 $\mu\text{g}/\mu\text{L}^{-1}$ scavenging activities were found to be 98.12%, 78.85% for Ascorbic acid, gum extract of *Commiphora wightii* respectively. The compounds scavenging activity which is the measure of antioxidant property at the concentration of above compounds at 200 $\mu\text{g}/\mu\text{L}^{-1}$ follows the order: Ascorbic acid > gum extract of while at higher concentration the same order is followed by gum stem extraction exchanged their position. The present study also reports Similar findings were reported in medicinal plants like ^[18, 10].

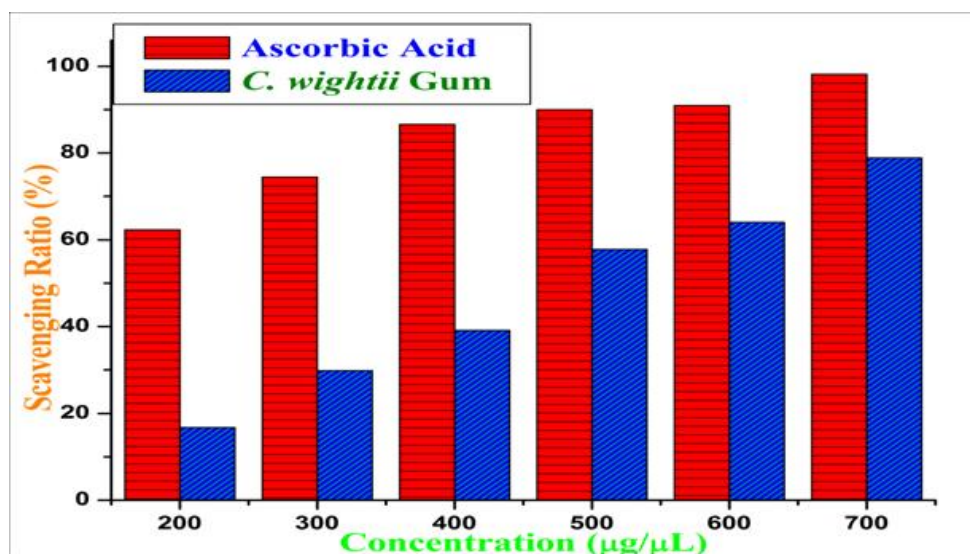


Fig 2: Radical-scavenging activity of the gum extract of *Commiphora wightii* on DPPH radicals (%)

Conclusion

Commiphora wightii is an important endangered medicinal plant with a variety of ethnic medicinal uses. The present study describes the qualitative analysis of *C. wightii* shows the presence of bioactive compounds such as Alkaloids, Flavonoids, Phenols, Tannins, Steroids and Terpenoids were present in *C. wightii* except, Saponins and Carbohydrates. Antibacterial properties of the imine base and its solvent extract evaluated and presenting in indicted that the compounds are active in exhibiting antibacterial role like gum 0.4, 0.4, 0.6 in gram negative bacteria and gum 0.4, 0.3, 0.6 in gram positive bacteria. Study confirms the antibacterial activity of gum extract of *C. wightii* the extract found effective bacterial strain, the activity of gum extract antibacterial activity higher than in gram negative bacteria, gum had a broad spectrum antibacterial activity of compare to in gram positive bacteria is a plant with a variety of ethnic medicinal uses. Antioxidant activity by inhibiting DPPH free radicals which indicates the gum extract is very much of *C. wightii* can be used as an accessible source of natural antioxidant agent. This is valuable information for preparation of drugs in pharmaceutical industry and stresses the need for more intensive research since they play a great role in healthcare

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