bark of *J. regia* stem was collected in July from Kulgam district in Jammu and Kashmir.

Plant material processing

The plants collected were authenticated with farm receipt no. 048 and book no. 2915. The bark was surface sterilized with mercuric chloride and dried in shade for 14 days. The dried bark was then crushed into a powdered form and extracted with methanol for 2 days using orbital shaker method. The extracted material was separated using Whatman filter paper no.1, the methanol was evaporated in a water bath at temperature <40°C, and then, crude extract was kept in a refrigerator at 4°C for further use.

Antibacterial activity

The antibacterial property of plant extract was determined using well diffusion assay [10]. The 0.5 McFarland was used as a standard (1.5×10^8 cells/ml) to adjust the turbidity of subcultured microorganisms. Mueller-Hinton agar plates were prepared and test microorganisms were seeded on the plates by spread plate process. After incubating for 30 min at room temperature, wells were punched with the help of borer. The dried extract was mixed with dimethyl sulfoxide (DMSO) and 50 µl of the extract was added to the wells. The standard was prepared for an antibiotic, chloramphenicol (HiMedia) as a positive control. DMSO was also added in a well as negative control to confirm that DMSO does not possess any antibacterial activity. After 24 h of incubation, the inhibition zone was measured and compared with an inhibition zone of the standard antibiotic used.

Table 1: Inhibition zone of methanolic bark extracts of *J. regia* on oral bacteria (mm)

Bacterial strains	Inhibition zone of ME	Inhibition zone of positive control	Negative control
S. aureus (standard)	16.3±0.3	25±0	-
S. aureus (clinical)	16.6±0.6	26±0	-
P. aeruginosa (standard)	17.4±0.9	29±0	-
P. aeruginosa (clinical)	17.9±0.4	24±0	-
S. mutans (standard)	17±0.0	29±0	-

S. aureus: Staphylococcus aureus, P. aeruginosa: Pseudomonas aeruginosa, S. mutans: Streptococcus mutans, J. regia: Juglans regia, -=No zone

Table 2: Preliminary phytochemical screening of methanolic bark extract of *J. regia*

Phytoconstituents	ME of *J. regia*
Flavonoids	+
Phenols	+
Alkaloids	−
Terpenoids	+
Cardiac glycosides	+
Saponins	+
Amino acids	−
Carbohydrates	+
Tannins	+
Fixed oils and fats	−
Steroids	−
Phlobatannins	+

+: Indicates the presence of phytoconstituents. −: Indicates the absence of phytoconstituents in ME of *Juglans regia*. ME: Methanolic extract, *J. regia: Juglans regia*

Table 3: TPC and TFC of methanolic bark extract of *J. regia*

Sample	TPC	TFC
J. regia	43.35±0.079 mg/g	17.28±0.125 mg/g

J. regia: Juglans regia, TPC: Total phenolic content, TFC: Total flavonoids content

Phytochemical screening

Methanolic extract (ME) was screened for the detection of phytoconstituents. Results of phytoconstituents screening are summarized in Table 1. Phytochemical screening was performed in the test tubes. The observations (Table 1) confirmed the presence of phytocomponents such as phenols, flavonoids, terpenoids, tannins, glycosides, phlobatannins, and cardiac glycosides.

Total phenolic estimation

In alkaline medium, the reaction of phenols with the phosphomolybdic acid of Folin–Ciocalteu reagent takes place and forms blue-colored complexes that are determined spectroscopically [11]. The overall phenols present in the sample were confirmed by a method involving Folin–Ciocalteu reagent (oxidizing agent) and gallic acid (standard). A standard gallic acid solution with a concentration of solution as 1 mg/ml or 1000 µg/ml was prepared. Serial dilution was performed to get different concentrations of reference gallic acid. In another test tube, 0.1 ml of the sample was added. Then, 1.5 ml of Folin–Ciocalteu (1:1 ratio) reagent and 1.2 ml Na_2CO_3 (7.5%) was transferred. In each test tube followed by incubation at 25°C for 20 min and finally absorbance was taken at 765 nm. Using different concentrations, a standard curve was drawn from which the amount of phenols in the sample was calculated and expressed as mg/g.

Total flavonoid content (TFC) estimation

The $AlCl_3$ colorimetric method was performed to know the concentration of flavonoids in the sample [12]. In this method ($AlCl_3$ colorimetric method), acid stable compounds are formed by $AlCl_3$ with C-4 keto and C-5 hydroxyl groups of flavonoids. Studies have described quercetin to be proper standard to determine the concentration of flavonoids in a sample extract. Hence, quercetin solution with different concentrations was taken to create the calibration curve. 10 mg of quercetin was mixed in 10 ml of methanol and then diluted to different concentrations of 20, 40, 60, 80, and 100 µg using methanol. The assay was performed using 0.1 ml of extract from stock solution (mg/ml), 0.2 ml of 5% sodium nitrate was added in each test tube. After 5 min, 0.2 ml of 10% $AlCl_3$ was added to the mixture, and finally, after 6 min, 2 ml of 1 M NaOH was added. Absorbance was taken at 510 nm. A standard curve with several concentrations of quercetin was drawn on the basis, of which concentration of phenols in the sample was calculated and expressed as mg/g.

Free radical scavenging activity

The free radical scavenging property of extract was calculated using 2,2-diphenyl-1-picrylhydrazylhydrate (DPPH) assay [13]. Using

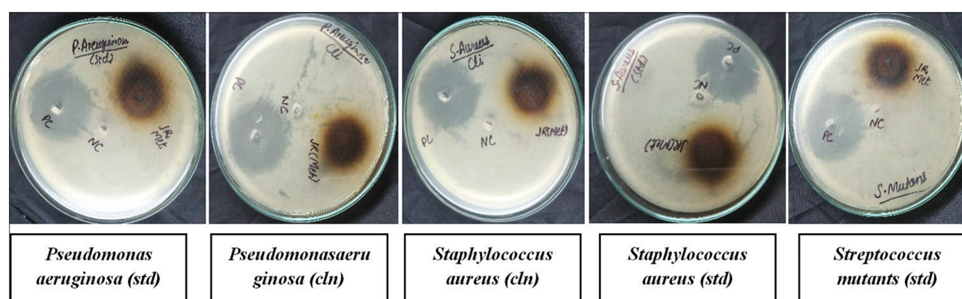

Fig. 1: Inhibition zones of methanolic extracts of *Juglans regia* against oral bacteria

methanol, 0.004% w/v of DPPH solution was prepared using methanol. Stock solution (1 mg/ml) of the sample and ascorbic acid were made using methanol. Several concentrations of sample and ascorbic acid were prepared in test tubes and 2.5 ml DPPH was added and tubes were kept in dark for 30 min. After incubation, the absorbance was taken at 517 nm using a spectrophotometer. A control sample was prepared using methanol and DPPH. Methanol was taken as a blank.

$$\%\ of\ scavenging = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

Statistical analysis

All the results data have been expressed in the form of mean ± standard deviation.

RESULTS

Antibacterial activity

Antibacterial activity of ME of *J. regia* was investigated and the results revealed different inhibition zones as shown in Table 1 and Fig. 1. The result showed that the ME was found to be potential antibacterial agents against the oral pathogenic bacteria which are responsible for different types of oral infections.

Phytochemical screening

The result revealed that MEs of *J. regi*a contain flavonoids, phenols, terpenoids, cardiac glycosides, saponins, and tannins, whereas carbohydrates, alkaloids, fats, and steroids were found absent. Preliminary phytochemical screening results were described in Table 2.

Total phenols and flavonoids estimation

Using standard curve equation of gallic acid and quercetin equivalents, the total phenolic content (TPC) and TFC of methanolic bark extract of *J. regia* was calculated and was found to be 43.35±0.079 mg/g and 17.28±0.125 mg/g, respectively (Figs. 2 and 3, Table 3).

Free radical scavenging activity

The free radical scavenging activity of methanolic bark extract of *J. regia* was evaluated using DPPH. It was seen that the scavenging activity of DPPH increased with the increase in concentration for both standard ascorbic acid and methanolic bark extract of *J. regia* interestingly (Fig. 4).

DISCUSSION

The main agent responsible for gum inflammation and dental cavities is a dental plaque. Some people avoid the use of chemical mouth rinse due to side effects such as tooth discoloration, change in taste, and formation of resistant microbes [14]. Hence, currently, the researchers are looking for medicinal plants to eliminate different kinds of infections. The current study showed that *J. regia* has a capability to serve as a potential antibacterial agent and thus can be used in oral hygiene products. According to our study, we indicated that ME of *J. regia* was effective in inhibiting the three bacteria *S. mutans*, *S. aureus*, and *P. aeruginosa*. The inhibition zones are shown in Table 1 and Fig. 1. Mohammed, 2012, reported the inhibition effect of ME of *J. regia* bark against *S. aureus, S. mutans*, and *P. aeruginosa* [15] which were less effective as compared to our methanolic bark extract. The antibacterial property possessed by the plant is due to the presence of phenolic compounds, flavonoids, tannins, and terpenoids [16]. Phenols are very essential plant components due to their capability to scavenge free radicals as they contain hydroxyl group groups in their structure; therefore, plant phenols may contribute directly to their antioxidant potential. Some studies were performed to know the amount of phenolics present in the bark extract of *J. regia.* Asha *et al.*, 2010, reported that the presence of total phenols and flavonoids in bark extract of *J. regia,* the TPC and TFC, was 20.32 mg/g and 11.48 mg/g, respectively, of dry weight [17]. On the other hand, Ogunmoyole *et al.,* 2011, reported the values of TPC and TFC ranging from 35.22±0.75 mg/g to 20.2±0.12 mg/g [18]. Interestingly, the result indicates higher phenolic and flavonoid content in our sample too

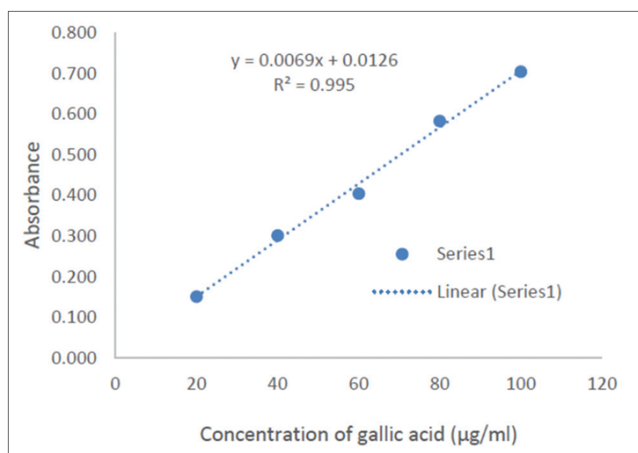

Fig. 2: Graphical representation of the standard curve of gallic acid

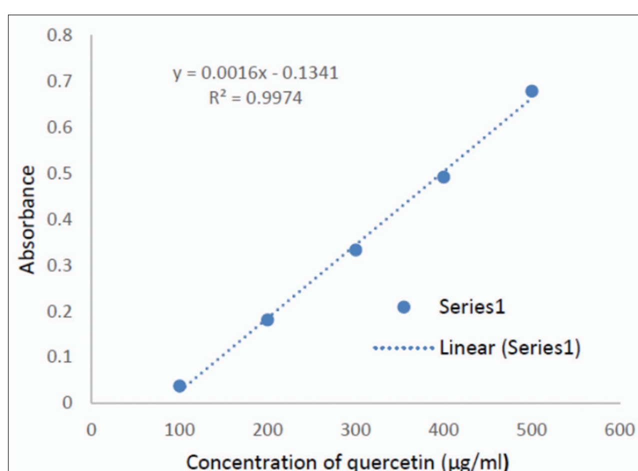

Fig. 3: Graphical representation of the standard curve of quercetin

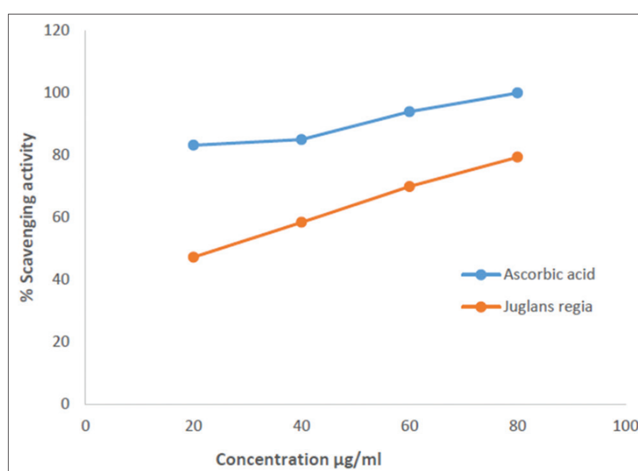

Fig. 4: DPPH scavenging activity of standard ascorbic acid and methanolic bark extract of *Juglans regia*

(43.35±0.079 mg/g and 17.28±0.125 mg/g). The difference may exist due to environmental factors, genetic factors, and date of sampling.

Scavenging process of free radical is an important mechanism in which antioxidant components reduce the oxidation of lipid. DPPH which acts as free radical has been currently used for the estimation of radical scavenging activity of different compounds. The free radical

DPPH which dissolves in methanol has purple color and defined absorbance at 517 nm. When antioxidant donates protons to DPPH, the purple color of the solution changes to yellow color with the decrease of absorbance [19]. The absorbance is inversely proportional to free radical scavenging activity. The scavenging activity of *J. regia* bark extract was found to be greater at a low concentration ranging from 20 to 80 μg/ml (47.16–79.31%) while Kshitij *et al*, 2012, have reported at higher concentration of 50–500 μg/ml (20–80%).

CONCLUSIONS

The results obtained in the study clearly show that *J. regia* bark may be considered a good candidate for employment as an effective antimicrobial agent against oral bacteria which can cause many oral diseases. To elevate oral sanitation, medicinal plant-based mouthwash can be used as a mediator and acts as a part of efficient home care medication. *J. regia* bark may also be included as a good source of healthy compounds such as phenols and flavonoids, suggesting that it could be useful in the prevention of diseases in which free radicals are present.

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CONFLICTS OF INTEREST

The authors declare that they no conflicts of interest.

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