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Potential Antimicrobial Efficacy and Phytochemical Screening Of Oleoresin Extract From Chir-Pine Against Bacterial Pathogens

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Abstract: In present study, antimicrobial, antioxidant and phytochemical activities of Oleoresin from Chir-pine was carried out with the help of Agar well diffusion method. The antimicrobial activities of different concentrations of Oleoresin against *Staphylococcus aureus, Escherichia coli, K. pneumoniae, Bacillus paramycoides, Enterobacter aerogens, Corynebacterium striatum, Pseudomonas aeruginosa* at two different concentrations Viz. 150 mg/ml, and 200 mg/ml were tested. On the other hand, antioxidant activity of Oleoresin was also tested. High antimicrobial activity was recorded at 200 mg/ml concentration of extract with the inhibitory zone of 24± 0.34 cm against *Pseudomonas aeruginosa* whereas minimum activity was recorded against *Bacillus paramycoides* at 200 mg/ml with the inhibitory zone of 11± 0.30 cm whereas at 150 mg/ml concentration of extract minimum zone of inhibition was recorded against *Pseudomonas aeruginosa,* (11± 0.35 Cm), *Bacillus paramycoides* (11± 0.30 Cm), *Escherichia coli* (11 ± 0.35 Cm). Phytochemical screening revealed the presence of Carbohydrates, Phenolic compounds, Steroid along with terpenoid and absence of Saponin glycosides, Flavoniodes, Insulin and Amino acid. Further significant amount of Rosin %, α - Pinene, β - Pinene , Carene and Turpentine Oil % was present in Oleoresin obtained from Chir-pine which provides significant antimicrobial activity against the bacterial pathogens.

Key words: oleoresin • medicinal plants • antimicrobial activity • phytochemical screening

Introduction

Indian Himalaya is an important repository which harbors rich germplasm with around 3,500 medicinal plant species. As per an estimate, Indian forest produces more than 80% of medicinal and aromatic plants. Due to rich floral wealth, India is emerging an integral part of global herbal market and could become largest raw material supplier of herbal drugs (Parveen, 2013). Plants have been traditionally used against various pathogens directly or indirectly as folk remedies in various medicinal systems along with modern medicine system (Alok, 1991 and Kala et al., 2004).

In Himalaya, different plants have been used in various diseases indicating their strength of herbal based industry in the area (Kala et al., 2004). Indian Himalaya, are endowed with huge diversity of medicinal plants which are still being used in herbal medicine. Uttarakhand Himalaya is well known for its biodiversity and cultural mosaic which supports huge diversity of medicinal plants. Chir-pine is a timber cum medicinal plants which have been traditionally used as a source of wood and its resin for various purposes. The knowledge on folk medicine has referred generation after generation but at the same time the scope of folk

medicine decreases due to rapid changes in the medical science. At the same time, due to the side effect of allopathic medicine, people attracted towards the use of ethnomedicinal plants. Utlilization of plants continuously at alarming rate is one of the most of important problems at recent time. On the other hand, using the plants in an unsustainable manner led to the natural extinction of the species in different areas of the globe (Sharma *et al*., 2012).

Plant based products has been used as source of medicine from millions of year ago by human beings. Now new complementary products have been obtained from the plants by various scientific technologies through proper microbiological and pharmacological based study (Akash *et al*., 2020b, Sarin, 1996). Presently, antimicrobial products obtained for treating various human ailments. So plants are the source of drugs in modern medicine system. Plants provides various nutraceuticals, food supplements, nutraceuticals and different pharmaceuticals which has huge efficacy in disease treatment (Akash *et al*., 2020a, Girach *et al*., 2003; Khanahmadi *et al*., 2010). At the same time, knowledge obtained from folk medicine or traditional medicinal system has lead to the new drug development knowledge (Qureshi *et al*., 2009). As the synthetic drugs causes adverse effect on human health so strong restriction should be followed while using a plant in any particular disease. So due to the adverse effects of the synthetic drugs, the trend of using old traditional system of medicine has increased in past few years (Knight, 1997). The antimicrobial and phytochemical as well as the antioxidant product of medicinal plants has made significant contribution in disease prevention (Drehe, 1996, 2007, Madhuri *et al.*, 2012). There we have made an attempt to study the antimicrobial, phytochemical screening along with the antioxidant property of Oleoresin**.** Oleoresin produced turpentine and rosin which are used widely as a cleaning agent, varnishes and paints, perfumes, synthetic rubber as well as the other material (Lee et al., 2001; Kelkar et al., 2006). The production of production can be affected by various factors Viz., light, temperature, and moisture content resulting into the emission of oleoresin yield (Dudareva et al., 2004; Martin et al., 2003). In *Pinus*, resin is produced and complex network radial and axial resin duct is responsible for its storage and through which it flow (Vazquez-Gonz ´ alez ´ et al., 2020). The formation of rein ducts in *Pinus roxbughii* is mainly affected by various environmental factors like soil, climate and genetic factors (Larson, 2012). Pinenes an important component is also act as a natural antifungal agent for treating various fungal diseases (Chang et al., 2008; Matan et al., 2012).

Materials and methods

Oleoresin was collected from Narendranagar forest division which under Garhwal Himalaya (Table. 1). The Oloeresin was pure as it is extracted by one novel method called 'Bore-hole method. Bore-hole was implemented as trial base in Narendranagar forest division. *Pinus* forest was divided into different diameter ranges 20-30, 30-40, 40-50, 50-60, 60-70 and 70-80 cm. Hand driven drill bits of 1.00 inch, 1.25 inch were made for drilling bore-hole. The chemical stimulant was sprayed and small pipes were fixed tightly in the holes which are attached with the plastic bags on which the oleoresin collected.

Table 1. Description of the plants

S. No	Botanical name	Common name	Family	Part used
1	*Pinus roxburghii*	Chir-pine	Pinaceae	Oleoresin

Tested Microorganism

In present study, *Staphylococcus aureus* (MTCC No. 1144), *Escherichia coli* (MTCC No. 118), *K.pneumoniae* (MTCC 4030), *Bacillus paramycoides* (MTCC OKO73274), *Enterobacter aerogens* (MTCC OK285220), *Corynebacterium striatum* (MTCC OK285222), *Pseudomonas aeruginosa* (MTCC 2474) at two different concentration Viz. 150 mg/ml, and 200 mg/ml concentration of Oleoresin was used in testing on mullarhinton agar plate. Al tested pathogens were procured from IMTECH,Chandigarh, India.

Antimicrobial activity: The antibacterial activity test was carried out using the disk diffusion method (Kaul, 1997).

Antioxidant Activity

DPPH Free Radical Scavenging Assay

Oleoresin extracts of the Pine was evaluated for their antioxidant power by the DPPH method according to standard methodology with some modification. Briefly, 1ml of the extract (at varying conc. in DMSO) was mixed with 0.004% DPPH solution and leave at room temperature for 1 hr. After 1 hr absorbance was measured at 517 nm by using double beam UV-Visible spectrophotometer. The change in the color from pink to yellow is directly proportional to the scavenging of the DPPH radical. Gallic acid was used as standard.1 ml of the solvent and 3 ml of the DPPH solution serve as blank.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of Blank} - \text{Absorbance of sample}}{\text{Absorbance of Blank}} \times 100$$

Phytochemical analysis

Oleoresin was tested for Phytochemical analysis in which major phytoconstituents were recorded by using standard method (Bauer *et al*., 1966; NCCLS, 1990). All the plant extracts were screened for the presence or absence of biologically active compounds such as Tanin, Saponin, Steroid Flavonoid, Terpenoid, Napthoquione, Phenol, Carbohydrate, Carbohydrate and starch.

Result

Antimicrobial activity: In present study, the antimicrobial activity of Oleoresin from Chir-pine was tested against seven different bacteria Viz. *Staphylococcus aureus, Escherichia coli, K.pneumoniae, Bacillus aramycoides, Enterobacter aerogens, Corynebacterium striatum, Pseudomonas aeruginosa* at two different concentration Viz. 150 mg/ml, and 200 mg/ml.), (Fig. 1). It was observed that antimicrobial potential of Oleoresin was active against all the seven tested bacterial culture which showed its huge activity with different inhibitory zone of inhibition. We have used DMSO as a solvent for preparation of 150 mg/ml, and 200 mg/ml of extract concentration. High antimicrobial activity was recorded at 200 mg/ml concentration of extract with the inhibitory zone of 24± 0.34 cm against *Pseudomonas aeruginosa* whereas minimum activity was recorded against *Bacillus paramycoides* at 200 mg/ml with the inhibitory zone of 11± 0.30 cm whereas at 150 mg/ml concentration of extract minimum zone of inhibition was recorded against *Pseudomonas aeruginosa,* (11± 0.35 Cm), *Bacillus paramycoides* (11± 0.30 Cm), *Escherichia coli* (11 ± 0.35 Cm), (**Table 2**).

Table 2. Antimicrobial activity of Oleoresin against the tested pathogens (Zone of inhibition in Cm)

Tested microorganism	200 mg/ml	150 mg/ml	Negative control (DMSO)
Staphylococcus aureus	23 ± 0.30	14 ± 0.30	-
Escherichia coli	18 ± 0.34	11 ± 0.35	-
K. pneumoniae	21 ± 0.30	14 ± 0.35	-
Bacillus paramycoides	11± 0.30	11± 0.30	--
Enterobacter aerogens	-	-	-
Corynebacterium striatum	-	10± 0.30	-
Pseudomonas aeruginosa	24± 0.34	11± 0.35	-

The antimicrobial activity of plant extracts affected by the permeability barrier of the membranous structure of bacteria resulting into the different zone of inhibition**.** It was also recorded from present study that, at lower concentration, lesser antimicrobial compound resulting into the low activity. Although in present study, we have tested all the plant extract of 150 mg/ml andm200 mg/ml against the selected pathogens.

In present study, the antimicrobial activities in 150 mg/ml concentrations of Oleoresin extract was active against different bacteria like *Staphylococcus aureus, Escherichia coli, K.pneumoniae, Bacillus aramycoides, Corynebacterium striatum, Pseudomonas aeruginosa* with the inhibitory zone of 14 ± 0.30 Cm, 11 ± 0.35 Cm, 14 ± 0.35 Cm, 11± 0.30 Cm, 10± 0.30 Cm and 11± 0.35. There was no activity recorded against *Enterobacter aerogens* at 150 mg/ml concentrations of Oleoresin extract. It was further recorded that, at 200 mg/ml concentration of extract was active against *Staphylococcus aureus, Escherichia coli, K.pneumoniae, Bacillus aramycoides, Pseudomonas aeruginosa* with the inhibitory zone of 23 ± 0.30 Cm, 18 ± 0.34 Cm, 21 ± 0.30 Cm, 11± 0.30 Cm and 24± 0.34 Cm whereas no activity was recorded against *Enterobacter aerogens* and *Corynebacterium striatum.*

The antimicrobial activities was tested in 150 mg/ml and 200 mg/ml concentrations of Oleoresin was determined against different bacteria like *Escherichia coli, Bacillus amyloliquefaciens, Pseudomonas aeruginosa, Staphylococcus aureus.* (**Fig.** 3, 4, 5), All the zone of inhibition of plant extract was recorded against the bacterial strains in mm and Gentamycin as positive control for bacteria. It was further observed that that DMSO extract of Oleoresin also showed different activities at both the concentrations for all the tested pathogens

Phytochemical screening of Oleoresin

The phytochemical investigation of Oleoresin reveals the presence of Carbohydrates, Phenolic compounds, Steroid along with terpenoid in the extract. Further, Rosin %, α - Pinene, β - Pinene , Carene and Turpentine Oil were recorded major compounds from the extract of Oleoresin of Chir-pine. On the other hand, phytochemicals like Saponin glycosides, Flavoniodes, Insulin and Amino acid were absent. (**Table 3**).

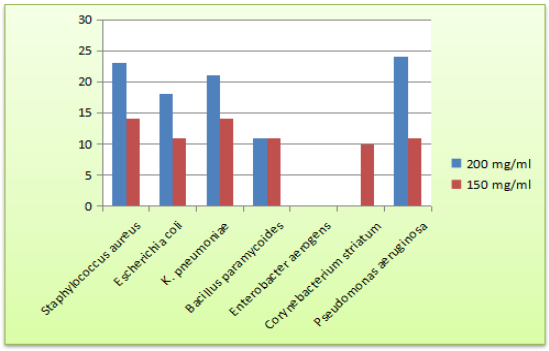


Figure 1. Antimicrobial efficacy of Oleoresin against the tested pathogens

Table 3. Phytochemical Screening of Oleoresin obtained in Bore-hole method

Test	Inference
Froth formation (Saponin glycosides)	-
Carbohydrates	+
Phenolic compounds	+
Flavoniodes	-
Insulin	-
Amino acid	-
Steroid and terpenoid	+

DPPH Free Radical Scavenging Assay

DPPH table data shown in Table 3 which depicts the results of DPPH free radical scavenging assay. Results are represented in terms of IC50 value which is the concentration of the drug to scavenge the 50% of the DPPH free radical. IC 50 value of the Chir-pine is found with IC50 =15.440 ± 0.131 µg /ml with moderate antioxidant activity as compared to the standard Gallic acid (IC50 =18.865 ± 0.075 µg/ml. Free radicals are generally harmful for human health. For authenticity of the method, we have used Gallic acid as standard which is an excellent antioxidative agent. Chir-pine and ascorbic acid have given dose dependent response i.e., as the concentration of Chir-pine and ascorbic acid increases the percentage of inhibition of DPPH radical also increases. (Fig. 2), (Table 4).

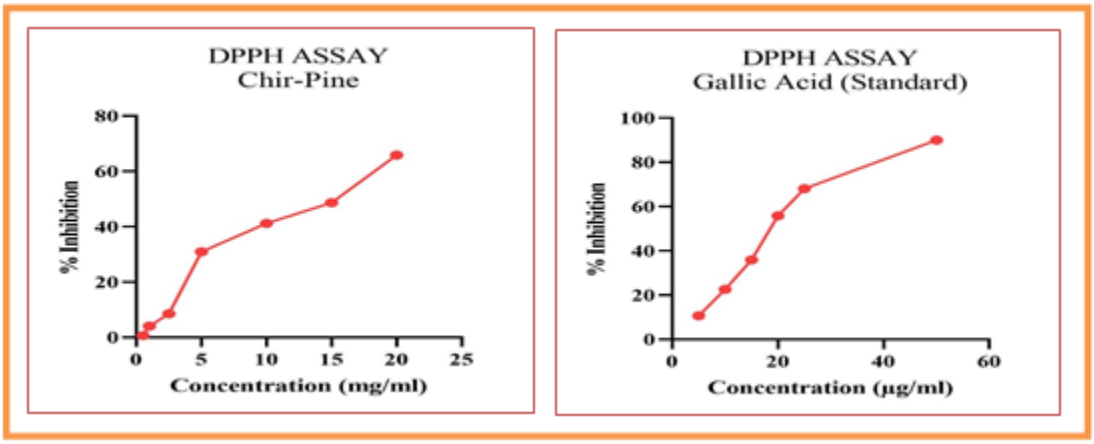
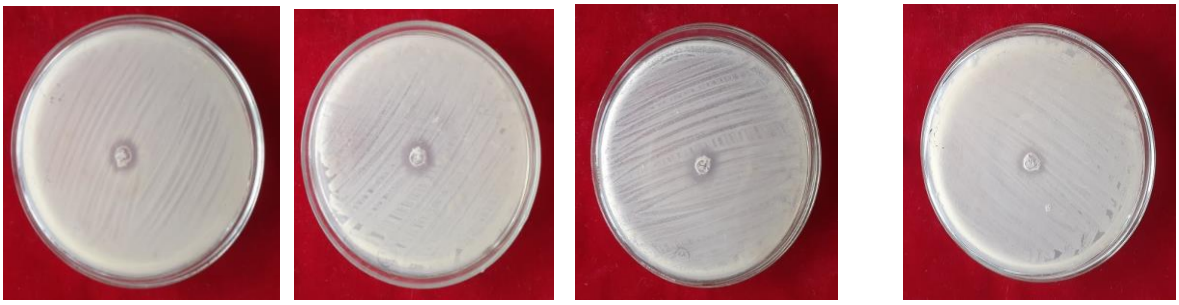


Figure 2. Variation % inhibition against concentration Chir-pine and Ascorbic acid (Standard)

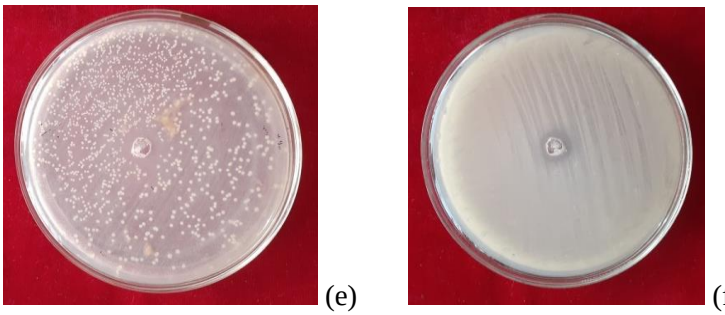
Table 4. DPPH assay of Oleoresin with standard as Gallic acid

Plant resin / Standard	IC50
Chir-Pine	15.440±0.131(mg/ml)
Gallic acid (Standard)	18.865±0.075 (µg/ml)



(a) **(b)** **(c)** (d)

Figure 3 Antimicrobial potential through disc diffusion assay showing the zone (150 mg/ml concentrations l) (a). *E.coli* (b). *S. aureus* (c). *Staphylococcus pyogenes* (d). *Enterobacter* or *Klebsiella aerogens*



(e) (f)

Fig 4. Antimicrobial potential through disc diffusion assay showing the zone (150 mg/ml concentrations l) (e). *Klebsiella pneumonia* (f). *Bacillus paramycoides*

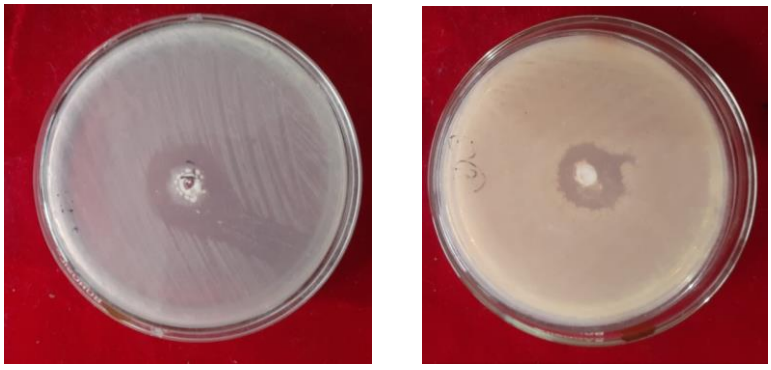


Figure 5. Antimicrobial potential through disc diffusion assay showing the zone (200 mg/ml concentrations l) (a). *Bacillus paramycoides* (b). *E.coli*

Discussion

Plants are the source of natural source of products which offers different antimicrobial property. They provide various antimicrobial agents and compounds which can cure various ailments. Due to the presence of various compounds in different parts of plants, they generally showed significant biological activity in different solvents. Further, the awareness of phytochemical is increasing due to their well impact against various disease of human beings . In present study, the phytochemical investigation of Oleoresin reveals the presence of Carbohydrates, Phenolic compounds, Steroid along with terpenoid whereas other tested phytochemicals like Saponin glycosides, Flavoniodes, Insulin and Amino acid were absent. On the other hand, Rosin %, α - Pinene,

β - Pinene , Carene and Turpentine Oil were recorded major compounds from the extract of Oleoresin of Chir-pine. It was revealed from the study that all the pines are capable of producing good quantity and quality of resin. Further, resin yield varied across the season (Hood and Sala, 2015; Lombardero, Neis et al., 2018 and Zas et al., 2020) . As per the study of Hood and Sala, 2015; Rodriguez-Garcia et al., 2013, groove-to-groove variation in resin yield is also affected by accumulation of induced response and wounds on the bark. It is imperative for the new generation to scientifically explore floral diversity, design constructive strategies for sustainable utilization and conservation of forest flora (Mir and Shafi, 2017). Since the existence of life, plants has played significant role for curing various diseases. As the development of human civilization occurred, the uses of medicinal plants also increase with their use in different ailments. It was about the 60,000 years ago, where the sumaerian clay salb which is equipment was used for the medicinal plants verification (Sumner, 2000). At present about 50% natural drugs being used for curing the various ailments. The description of maximum explored plants can be seen in charak samhita and traditional medicinal system. Plants like *Asparagus racemosus, Ocium santum, Withania somifera, Tinospora crdifolia, Rauwolfia serpentine* and so on has been well described in our ancient Ayurveda. At the same time the other system like Chineses, Unani also has been recognized for uses of medicinal plants. These plants are still being used in various diseases for their potential ability of ailments curing. Oleoresin from Chir-pine has huge activity due to the presence of various secondary metabolites like Carbohydrates, Phenolic compounds, Steroid along with terpenoid and Rosin %, α - Pinene, β - Pinene , Carene and Turpentine Oil provide huge antimicrobial efficacy to Oleoresin against the bacterial pathogens.

Today millions of people depend on plants for food, fodder, and for medication. At recent time various discovery is going on the status of medicinal plants. Various numbers of secondary metabolites like, steroids, alkaloids, essential oils and glycosides has been discovered from plant parts**.** Medicinal plants play essential role in curing various diseases so recent medicinal system is focusing on the novel drug discover on the basis of the data from folk medicinal system (Anwar *et al.*, 2013 and Das *et al.*, 2010, and Rahmoun *et al*., 2013). The result from present study indicated that DMSO extract of Oleoresin showed significant antimicrobial activity against *Staphylococcus aureus, Escherichia coli, K.pneumoniae, Bacillus aramycoides, Enterobacter aerogens, Corynebacterium striatum, Pseudomonas aeruginosa.* This could be due to presence of various phytochemicals compounds in the extract (Cowan, 1999). Similar finding was recorded by different workers with high antimicrobial activity of medicinal plants against the various pathogens (Aboaba *et al.*, 2001, Karou *et al*., 2006).

Western Himalaya possesses about huge wealth of vascular plants that which acted as source of natural remedies. It is imperative for the current generation to scientifically explore floral diversity, design constructive strategies for sustainable utilization and conservation of forest flora (Dar *et al*., 2002 and Mir and Shafi, 2017). Disc diffusion method is one of the best suited method for testing the antimicrobial property of medicinal plants. Workers like Negi *et al.*, 2013, Rao *et al*., 2007, Elzaeawely *et.al*., 2005, Jang *et. al.* 2005 have tested the activity of medicinal plants against various pathogens with the help of this method. All these works have recorded agents like phenolic substances in the extract which are responsible for significant antimicrobial activity against the pathogens. In present study, huge results of antimicrobial activity was recorded against *Staphylococcus*

aureus, Escherichia coli, K.pneumoniae, Bacillus aramycoides, Corynebacterium striatum, Pseudomonas aeruginosa. . High antimicrobial activity was recorded at 200 mg/ml concentration of extract with the inhibitory zone of 24± 0.34 cm against *Pseudomonas aeruginosa* whereas minimum activity was recorded against *Bacillus paramycoides* at 200 mg/ml with the inhibitory zone of 11± 0.30 cm whereas at 150 mg/ml concentration of extract minimum zone of inhibition was recorded against *Pseudomonas aeruginosa,* (11± 0.35 Cm), *Bacillus paramycoides* (11± 0.30 Cm), *Escherichia coli* (11 ± 0.35 Cm). The results from present study showed significant antimicrobial activity against all the tested seven tested pathogens which could be used for potential antimicrobial agent and disease causing natural product. Further more study need to be done so that it can be used as a natural drug while testing it for drug discovery.

Conclusion

The study has emphasized the Oleoresin extract at 100 mg/ml and 200 mg/ml against the seven most harmful bacteria which showed huge activity with the inhibitory zone. Significant results with maximum activity at 200 mg/ml concentration of extract of Oleoresin with the inhibitory zone of 24± 0.34 cm against *Pseudomonas aeruginosa* was recorded whereas minimum activity was recorded against *Bacillus paramycoides* at 200 mg/ml with the inhibitory zone of 11± 0.30 cm. On the other hand, at 150 mg/ml concentration of extract minimum zone of inhibition was recorded against *Pseudomonas aeruginosa,* (11± 0.35 Cm), *Bacillus paramycoides* (11± 0.30 Cm), *Escherichia coli* (11 ± 0.35 Cm). Presence of various phytochemicals like Phenolic compounds, Carbohydrates, and steroids could be good antimicrobial agents from Chir-pine against various pathogens.

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