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Antiplaque Activity of *Juglans Regia* L. and Characterization of Juglone from *Juglans Regia* L.

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Abstract: Problem statement: Oral hygiene has become the most important in current era. Due to growing need for aesthetic and hygeine oral hygiene has gained impetus. In this research paper we have studied new varieties of natural products which help in improving the oral hygiene. **Approach:** Two varieties of *Juglans regia* bark were extracted using hot and cold extraction methods and there *in vitro* antimicrobial activities were tested against four microorganisms related to dental caries (*Streptococcus mutans*, *Streptococcus sobrinus*, *Actinomyces viscosus*) which are known to be implicated in dental caries. **Results:** Both the varieties of *Juglans regia* showed good anti plaque activity. Kashmir variety of *Juglans regia* showed maximum Antiplaque activity. **Conclusion:** Natural products like *Juglans regia* can be used for improving oral hygiene and can be included in the products of oral hygein.

Key words: *Streptococcus mutans, Streptococcus sobrinus, Actinomyces viscosus, Juglans regia*

INTRODUCTION

Oral hygiene has gained importance in recent years. Antimicrobial agents are commonly incorporated into hygiene products for the treatment and prevention of plaque and gingivitis. The pathogenic organisms for plaque are *Streptococcus mutans*, *Streptococcus sanguins and Actinomyces viscous* (Svensater *et al*., 2003). Plants are known to have defense systems against pathogenic bacteria (Smith *et al*., 2005). Medicinal plants that are commonly used may be a good source for safe antibacterial agents (Sato *et al*., 2000). Walnut or *Juglans regia L*. plant found in Himalayan states of India is well known for its antioxidant potential (Labucka *et al*., 2008). Phytochemically *Juglans regia* L. contains naphthaquinones, tannins, flavonoids and tannic acids. The current study we report that the antiplaque activity of *Juglans regia* tested on the strains of *Streptococcus mutans, Streptococcus sanguins and Actinomyces viscous.*

Plant: Bark of two varieties of *Juglans regia* viz. Kashmir variety and Himachal variety was collected from Delhi and Mumbai local market. 3 batches were procured of 1, 2 and 5 kg of each variety of *Juglans regia*. The re-extraction was carried out in triplet. The authentication was carried out by Dr. Nayak at Nicholas Piramal.

Test materials: Successive and direct (Hot and cold extractions) extractions of both the varieties of bark were carried out and extractive values were noted. 100 gm of powdered bark of *J. regia* was extracted successive in Methyl acetate, Chloroform, Acetone, Ethanol, Hydroalcohol (HA) and Water (Fig. 1). The extracts were evaporated to dryness. Yields obtained are given in Table 1. Maximum yield is obtained from Kashmir variety of *Juglans regia* by direct cold method.

Chemical constituents of *Juglans regia* (Bark):
TLC: TLC was performed on pre-coated Silica gel Merck plates. Solvent system used chloroform and methanol. The plates were derivatized with Ferric chloride and alcoholic potassium hydroxide.

HPTLC: HPTLC was conducted at Anchrom. *Juglans regia* contains besides other compounds *Juglone* and *Quercetin*. These two compounds were obtained from Sigma-Aldrich and used as marker compounds for our profiling:

Sample 1:	$CHCl_3$ ext
Sample 2:	Acetone ext
Sample 3:	Ethyl acetate ext
Solvent system:	N-Hexane: Ethyl acetate (8:2).
Spraying reagent:	10% ethanol KOH

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Table1: Concentration of Juglone in various extracts of *Juglans regia* obtained from various sources

Type	PT	EA	CL	AT	ET	HA	W
Successive hot Himachal	0.57	0.72	0.92	2.25	6.23	10.16	15.2
Successive hot Kashmir	0.77	1.14	1.65	4.90	8.40	15.20	17.3
Direct cold Himachal	0.87	0.62	0.87	3.24	8.31	17.03	15.5
Direct cold Kashmir	1.66	1.97	1.40	5.90	1.31	18.76	15.0

Table 2: Activity of various extracts on *S. mutans, S. sanguins, A. viscous*

	Observed MIC (g mL)			
Extract	Diluent	*S. mutans*	*S. sanguins*	*A. viscosus*
Kashmir variety				
Acetone	Methanol	250	200	100
Chloroform	Ethyl acetatate	100	250	>1000
Ethyl acetatate	Ethyl acetatate	250	500	>1250
ethanol	DMSO	400	600	>1000
Himachal variety				
Hydroalcohol	DMSO	750	>1500	>1500
Aqueous	DMSO	>1500	>1500	>1500
Ethanol	DMSO	500	>1500	>1500

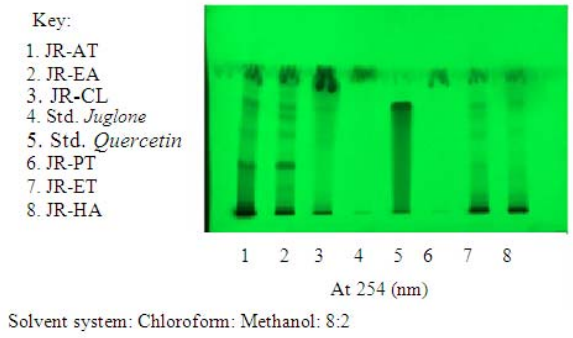

Fig. 1: HPTLC data of the extracts in chloroform methanol system

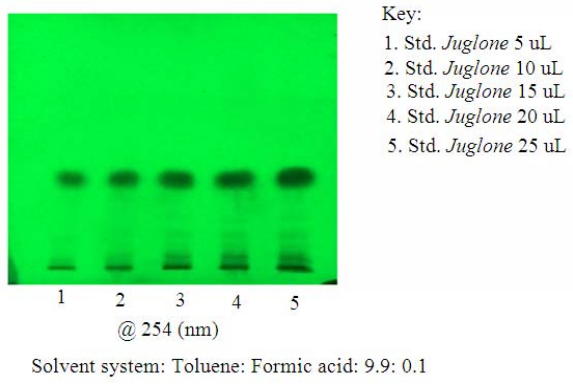

Fig. 2: HPTLC data of the extracts in Toluene: Formic acid system

In Fig. 2 presence of *Quercetin* is shown in all extracts as it matches with Rf of Std. *Quercetin. Juglone* has moved to the solvent front as solvent system as solvent system is more polar but shows presence in all extracts. Different concentrations of Std. Juglone was loaded on a plate for linearity so that concentration of Juglone can be calculated in various extracts.

Antimicrobial activity: The antimicrobial activity of *Juglans regia* was tested. *Juglans regia* was tested for anti plaque activity using microbial activity. The extracts already prepared were tested for antimicrobial activity against target microorganism viz. *Streptococcus mutans, Streptococcus sanguins and Actinomyces viscous.*

MATERIALS AND METHODS

The antimicrobial activity was tested using Agar Steark Plate method. Sterile Tryptone soya agar plates containing a range of concentration of the active were prepared. The one-day test cultures were streaked on the surface using a sterile L spreader. Sixteen-hour-old cultures were used when testing the antimicrobial activity with density adjusted to 1.5×108 cells mL^{-1} (Mc Farlands standard) using sterile saline. The plates were incubated at 37C for 48 h. MIC was recorded as lowest concentration of the active that does not allow microbial growth.

RESULTS AND DISCUSSION

Anti microbial activity was seen in agar streak plate method. The study suggested that Kashmir variety of *Juglans regia* showed maximum antimicrobial activity. It was also observed that Chloroform extract showed highest antimicrobial activity (Table 2).

CONCLUSION

The current studies shows that natural products like *Juglans regia* are potential antimicrobial agents and can be used in oral hygiene products.

The natural products should be explored more for its antimicrobial activity and isolation of the active ingredients should be carried out to extensively study such products and its activity.

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