Journal of Clinical & Experimental Pathology

ISSN: 2161-0681

Shinde, et al., J Clin Exp Pathol 2023, 13:4

Research Article Open Access

Formulation and Phytopharmacological Activity Studies of Fresh Juice of *Acacia arabica* Stem and Leaves for the Treatment of Variety of Dental Problems

Shinde SA[1], **Chavhan SA**[1*], **Bute SD**[2] **and Rangari VD**[3]

[1]*Department of Pharmaceutical Sciences, Rajendra Gode College of Pharmacy, Buldana, India*

[2]*Department of Pharmaceutical Sciences, JL Chaturvedi College of Pharmacy, Nagpur, India*

[3]*Department of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya, Chattisgarh, India*

Abstract

It is well known that use of plant material for oral healthcare and treatment of periodontal disorder is common in many cultures and many of such remedies are very effective with respect to long term health. From literature review, it comes to know that *Acacia arabica* stem is used as chewing stick and claimed to be useful for health of gum. The objective of the proposed study is to perform the phytochemical studies on the fresh juice of babul stem and leaves. It is further envisaged to study anti-inflammatory, analgesic and antimicrobial properties of the dried fresh juice. Objective shall further be extended to convert the dried fresh juice to a suitable formulation for the treatment of variety of dental problems. Phytochemical tests suggest presence of carbohydrates, steroids, tannins and flavonoids in leaf and stem juice both. Leaf juice at the dose of 200 mg/kg bodyweight was found to be very effective in imparting analgesic effect. In the anti-inflammatory studies leaf juice at the doses of 50 mg/kg, 100 mg/kg and 200 mg/kg body weight was effective to reduce inflammation. The activity of leaf juice was more than that of stem juice but both can be claimed to have analgesic and anti-inflammatory activity. The activity may be due to presence of tannins, steroids and flavonoids. The dry juice was incorporated into a mouthwash formulation at 1% leaf juice, 1% stem juice and 1% leaf and stem juice both of which formulation no. 3 with leaf and stem juice 1% both was better in taste, odour and colour.

Keywords: Anti-inflammatory; Analgesic; Mouthwash; *Acacia arabica*

Introduction

Herbal drugs constitute a major part in all the traditional systems of medicine. Herbal medicine is triumph of popular therapeutic diversity. Plants above all other agents have been used for medicine from time immoral because they have fitted the immediate personal need are easily accessible and inexpensive. Indian material medica includes 2000 natural products of therapeutic importance of which 400 are of mineral and animal origin and rest are of vegetable origin.

There are approximately 1250 Indian medicinal plants, which are used in formulating therapeutic preparation according to ayurveda and other traditional system of medicine. Indian medicinal plants used in different traditional system of medicine are studied in different universities and institutes; these works are still progressing and the results are reported in professional journals. The large herbal resources around the globe are being exploited more effectively (Table 1) [1].

Plant name	Purpose and method of use
Acacia catechu	Catechu paste is used to treat the bleeding gums and for tooth hypersensitivity
Acacia nilotica	Fresh twig is used as tooth brush for keeping the gums and teeth health and clean
Achyrathes aspera	Twig is used for brushing teeth to treat dental problem
Azadirachta indica	Fresh twig is used as tooth brush to prevent gum diseases and pyorrhoea
Aristolochia bracteolate	Root juice is applied to the site of toothache of relieving pain
Cinnamonum camphora	Tender twigs are chewed or the paste of stem bark is applied in aching teeth

Table 1: Plants used for oral healthcare in India.

The World Health Organization (WHO) estimates that about 80%of the population living in developing countries relies, almost exclusively on traditional medicine for primary healthcare needs. In almost all the traditional medicines, the medicinal plants play a major role and constitute the backbone of traditional medicine. There are various plants which are used for oral healthcare in India their names and uses are enlisted here.

***Corresponding author:** Chavhan SA, Department of Pharmaceutical Sciences, Rajendra Gode College of Pharmacy, Buldana, India; E-mail: sarinchavhan21@gmail.com

Received: 07Oct2019, Manuscript No. JCEP-23-3283; **Editor assigned:** 10Oct2019, PreQC No. JCEP-23-3283 (PQ); **Reviewed:** 24-Oct-2019, QC No. JCEP-23-3283; **Revised:** 14-Jun-2023, Manuscript No. JCEP-23-3283 (R); **Published:** 12-Jul-2023, DOI: 10.4172/2161-0681.23.13.456

Citation: Shinde SA, Chavhan SA, Bute SD, Rangari VD (2023) Formulation and Phytopharmacological Activity Studies of Fresh Juice of *Acacia arabica* Stem and Leaves for the Treatment of Variety of Dental Problems. J Clin Exp Pathol 13: 456.

From ethnobotanical information it is evident that babul is a popular drug used by people for fulfilling regular oral healthcare needs as a prevention or treatment of oral disease specially with an assumption that it improves the quality of gums and health of gums.

Materials and Methods

Major orodental diseases specially related to gums

Gingivitis: Gingivitis is an inflammation of the gums surrounding the teeth. Gingivitis is one of many periodontal diseases that affect the health of the periodontium (those tissues that surround the teeth and include the gums, soft tissues and bone).

Periodontal diseases are often classified according to their severity. They range from mild gingivitis, to more severe periodontitis and finally acute necrotizing ulcerative gingivitis, which can be life threatening [2].

Periodontitis: Periodontitis is the name of a collection of inflammatory diseases affecting the tissues that surround and support the teeth. Periodontitis involves progressive loss of the bone around teeth which may lead to loosening and eventual loss of teeth if untreated.

Swollen gums: Change of gums from a thin, well-adapted, continuous covering around the teeth to a thick swollen red mass, may not only appear unsightly, but also acts as a platform for further destruction of healthy teeth and supporting bone.

Bleeding gums: Bleeding gums is among the common conditions affecting the oral cavity. The Chinese might have noticed bleeding gums as early as 2500 BC. They termed the associated diseases as "Ya-Kon" which means diseases of soft tissue surrounding the teeth. This problem still continues to affect us even with so many modern facilities available in the field of oral care (Figure 1) [3].

Figure 1: *Acacia nilotica*.

Biological source: *Acacia nilotica Delile*

Subspecies: Indica (Benth.)

Family: *Mimosaceae*

Vernacular names: Babul, black babul, Indian gum arabic tree.

Description: A moderately sized, almost evergreen tree with a short trunk, a spreading crown and feathery foliage, found throughout the drier parts of India. The tree generally attains a height of 15 m and a girth of 1.2 m, though trees up to the height of 30 m and girth of 3 m have also been recorded [4].

Bark: Dark is brown to almost black, longitudinally fissured or deeply cracked.

Leaves: Leaves 2.5 cm-5 cm long, bipinnate with spiscent stipules, pinnules narrowly oblong.

Flowers: Flowers are golden-yellow, fragrant, crowded in long stalked globose heads, 1.5 cm in diameter, forming auxillary cluster of 2-5 heads.

Pods: Pods are flat, 7.5 cm-15 cm, contracted between the circular seeds. Babul is perhaps the most important tree of drier parts of India.

Varieties: There are three recognized varieties in India: 1) var. cuppressiformis Stewart (Ramkanta or ramkanti babul or kabulikikar) with its characteristics broom-like ascending branches. This variety is not much used; 2) var. vediana Cooke (kaora, kaulia, kauria or vedibabul) is a smaller variety with shorter bole and rough, fissured bark. The wood is inferior to that of telia babul and is usually considered fit only firewood; 3) Telia babul- the much-prized typical variety (goditeli or telia babul) with a spreading shady crown of feathery foliage and monoliform pods. This variety is most extensively grown in plantations or in natural forest crops. Babul is indigenous to the plains of Andhra Pradesh and Maharashtra and is cultivated or found self-grown through the drier parts of India, ascending to an altitude of 900 m. The bark from older trees, though richer in tannins, is likely to be of high colour [5].

Results

Collection of plant material

The plant material was collected from wildly grown mature trees along road sides and boundaries of farmyards. The full-grown trees of heights more than 3 m-4 m are chosen. All material collected from the fields around Nagpur city. Leaves and stem were collected separately.

Preparation of juices

The plant parts collected are immediately washed and stem was cut into small pieces. The stem pieces are weighed and transferred to mixer to make fine powder and to that measured quantity of water was added and again allowed the mixer to run for few minutes. Juice was separated by squeezing the material trough clean muslin cloth. The juice so obtained was filtered by vacuum filtration through sintered glass funnel so that all the green and suspended material gets removed [6].

This clear liquid was then allowed to dry in trays at normal temperature to avoid degradation of heat sensitive constituents that might be present in the juice, till all the water got evaporated and

complete dry powder was formed showing brown color. The dry juice was transferred to air tight glass container. This container was placed inside a vacuum container to avoid attack of moisture. The same procedure was applied for leaf juice. Percentage yield of dry juices was determined. Table 2 indicates the extractive value of stem juice and leaf juice.

Sample code	Weight of plant part taken (g)	Quantity of water added	Quantity of juice obtained (ml)	Weight of dry juice obtained (g)	% Practical yield	Average
AASJ-1	300	700	400	12.3	4.8	3.2175
AASJ-2	300	700	340	6.7	2.23	
AASJ-3	476	700	300	14	2.94	
AASJ-4	250	500	350	9	3.6	
AALJ-1	310	500	310	12	3.87	4.5675
AALJ-2	400	600	350	14	3.5	
AALJ-3	200	500	330	13	6.5	
AALJ-4	240	500	300	11	4.4	
Note: AASJ-*Acacia arabica* Stem Juice; AALJ-*Acacia arabica* Leaf Juice						

Table 2: Extractive values of juices.

Preliminary phytochemical screening of *Acacia arabica* willd stem and leaf juice

Phytochemical tests suggest presence of carbohydrates, steroids, tannins and flavonoids in leaf and stem juice both (Table 3).

Plant constituents	Test/Reagents	Leaf juice	Stem juice
Alkaloids	Hager's reagent	-	-
	Wagner's reagent	-	-
	Draggendorff's reagent	-	-
	Mayer's reagent	-	-
Amino acid	Ninhydrin test	-	-
Carbohydrates	Molisch's test	+	+
	Barfoed's test	+	+
Flavonoids	Shinoda test	+	+
Proteins	Biuret test	-	-
	Xanthoproteic test	-	-
Steroids	Salkowaski reaction	+	+
	Libermann-burchard test	+	+
Saponins	Foam test	-	-
Tannins	Ferric chloride test	+	+
	Lead acetate test	+	+
Note: +ve present; -ve absent			

Table 3: Preliminary phytochemical screening stem and leaf juice.

Thin layer chromatography

The stem and leaf juice was subjected to thin layer chromatographic studies, to find out the probable number of compounds present in it. A number of developing solvent systems were tried, but the satisfactory resolution was obtained in benzene: acetone: methanol (6:2:2). After development of plates, they were air dried (Table 4) [7].

Extract	Solvent system	No. of spot (R_f value)		
		UV light	Iodine	Vanillin sulphuric acid
Leaf juice of *Acacia nilotica*	Benzene:Acetone:Methanol (6:2:2)	1 spot (0.66) yellow fluorescence	1 spot (0.41)	3 spots (0.88), (0.66), (0.41)
Stem juice of *Acacia nilotica*	Benzene:Acetone:Methanol (6:2:2)	No spot	No spot	2 spots (0.84), (0.38)

Table 4: TLC of stem and leaf juice of *Acacia nilotica*.

Detection of the spots

The spots were sequenceally visualized by UV light (UV chamber), iodine vapors and spraying reagent (vanillin sulphuric acid). The Rf values of the spots were calculated (Figure 2) [8].

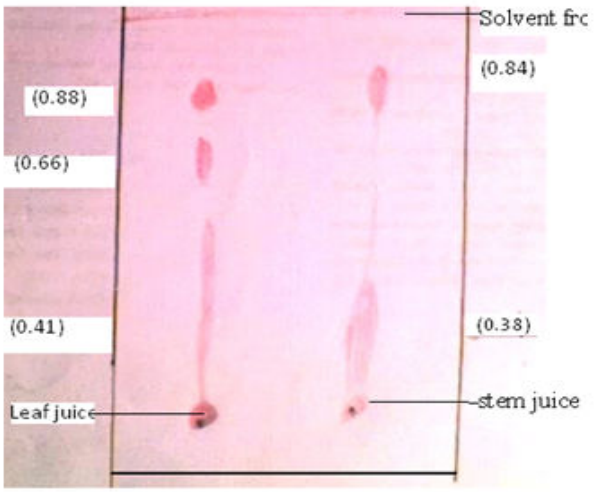

Figure 2: TLC of stem and leaf juice of *Acacia nilotica*.

Column chromatography

It was used for separation and isolation of different chemical constituents of the juices of *Acacia arabica* willd leaf juice. Fractionating column of size 65 cm × 3 cm and 30 cm × 1.5 cm was used for column chromatography.

Column chromatographic grade silica gel (60 to 120 mesh size) was used as an adsorbent and different solvents were used as eluents. The different solvents were distilled and demoisturised with anhydrous sodium sulphate prior to use (Table 5) [9].

No	Eluting solvent	No. of fractions	TLC pattern	Rf Value
1	Pure benzene	21	Two spots with bright white fluoroscence	0.3, 0.1
2	Benzene 95: Acetone 5	22	Two spots in UV white fluoroscence	0.35, 0.12
3	Benzene 92.5: Acetone 7.5	43	Two spots	0.24, 0.98
4	Benzene 90: Acetone 10	31	Two spots in UV	0.42, 0.1
5	Benzene 88.5: Acetone 12.5	24	One spot	0.3
6	Benzene 85: Acetone 15	191	Three spots	0.4, 0.56, 0.88

Table 5: Column chromatography.

Refractionation method

A white coloured matter was deposited in containers of fractions of benzene: acetone (85:15). This material was dissolved in alcohol and taken for refractionation. The column was packed similarly using benzene: petroleum ether (50:50). The same procedure was repeated for refraction using various combination of benzene: acetone (100%, 95:05 up to 85:15). The 22 fractions from RBA15%-19 to RBA15%-41 containing a white turbid material was mixed together and labeled as MRBA-41, washed with pet. Ether and chloroform and then deposited product has been sent for infra-red spectroscopy (Table 6) [10].

No	Eluting solvent	No. of fractions	TLC pattern
1	Benzene:Pet ether (50:50)	10	Single spot
2	Pure benzene	20	No spot
3	Benzene:Acetone (95:5)	32	No spot
4	Benzene:Acetone (90:10)	59	Two spots but no solid deposited
5	Benzene:Acetone (88.5:12.5)	20	No spot
6	Benzene:Acetone (85:15)	42	Three spots 0.19, 0.53, 0.86

Table 6: Refractionation by column chromatography.

Spectroscopic studies

Sample code: MRBA-41

Solvent system: Benzene: Acetone (85:15)

Characteristics: A solid material with whitish brown colour and strange odour.

TLC profile: A pink coloured single spot in 1% vanillin sulphuric acid at R_f value-0.21

Solubility: This isolated material was completely soluble in acetone, methanol and water and not soluble in benzene, petroleum ether and chloroform (Figure 3 and Table 7) [11].

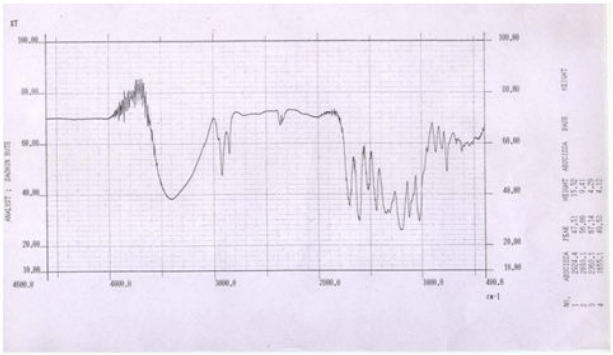

Figure 3: Infra-red spectra of MRBA-41.

Sr. No.	Wave No. (cm^{-1})	Interpretation
1	3410	OH stretching
2	2924.4	CH stretching
3	2853.1	CH stretching
4	1710.1	C=O stretching
5	1615.1	C-O stretching
7	1442.9	CH bending vibration methyl group
8	1360.8	C-N stretching
9	1205.1	C-O stretching vibration
10	1124.6	C-(C=O)-C stretching
11	1030.1	Aryl-O-CH_2 bending
12	864.2	-O-O- bending (peroxide)
13	758.1	Aromatic C-H bending
14	617.3	1-3 dioxalone bending

Table 7: Infra-red spectrum.

Interpretation of infra-red spectrum of sample no. MRBA-41

The IR spectrum of MRBA-41 showed the OH stretching at 3410 cm^{-1} in the form of broad hump. A typical CH stretching is observed at 2924.4 cm^{-1}. Another CH stretching is observed at 2853.1 cm^{-1}. A typical C=O stretching is observed at 1710.1 cm^{-1} indicates keto function in the molecule. C-O stretching at 1615.1 and 1205.1 was observed. CH bending vibration of methyl group at 1442.9. The 1360.8 cm^{-1} was indicative of the C-N stretching and peak 1124.6 is of CH_3CO unit. Peak at 1030.1 was of Aryl-O-CH_2 bending, while 864.2 suggest -O-O- bending of peroxides. Peaks 758.1 and 617.3 are of aromatic C-H bending and 1-3 diaxolone bending [12].

The compound has given positive Molisch's test. On the basis of spectral data and chemical tests, MRBA-41 seems to be a complex hydrolysable tannin compound. However further structural elucidation needs the 1HMR, 13NMR and mass spectral data which could not be taken due to paucity of material.

Acute toxicity studies

Procedure: The overnight fasted rats were weighed and divided into 6 group of six in each. Stem and leaf juice of *Acacia arabica* willd has been given in various doses (500 mg/kg-5000 mg/kg body weight) by oral route. After administration of the juice suspension, the animals were observed continuously for the 24 hours for the death due to acute toxicity [13].

Result: Mortality was not found till dose of 500 mg/kg-5000 mg/kg body weight in stem juice and leaf juice both.

Tail flick method

Studies were carried out on male albino rats of either sex weighing 100 g-150 g procured from JLCCP college of pharmacy animal house, India. All experiments were performed in accordance with our institutional animal ethics committee. Animals were used in five groups of six in each experiment except analgesiometer test, which were performed five groups. The animals were given standard pellet diet and tap water ad libitum.

Analgesiometer test

Six groups of five animals were taken. Tail flick response was evoked by placing rat tail over a wire heated electrically. The analgesiometer (Inco India) was adjusted at 2 ampere. The cut off period of 10 seconds was observed to prevent the damage to tail. The initial reaction time of each rat was recorded. The animals were treated with the test drug and standard, post treatment reaction time of each animal was determined at 30, 60 and 120 minutes from the administration of drug [14].

Statistical analysis

All the results were statistically analyzed by Anova and expressed as the mean ± S.E.M. A value less than 0.05 were considered significant (Figure 4 and Table 8).

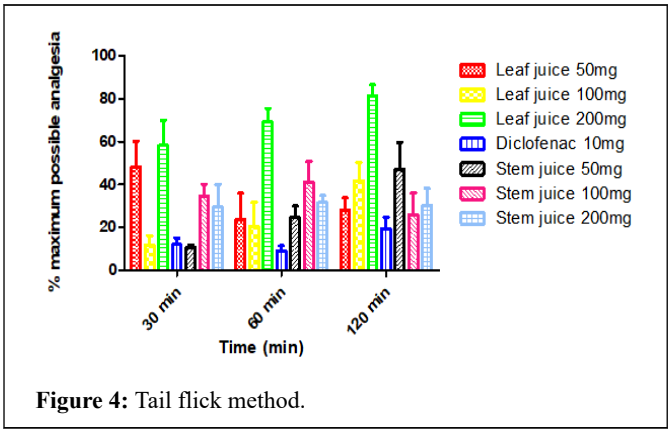

Figure 4: Tail flick method.

Dose	30 min	60 min	120 min
Standard	3.99 ± 0.28	3.595 ± 0.2	4.45 ± 0.4
Leaf 50 mg/kg	6.03 ± 0.58*	4.91 ± 0.84	4.90 ± 0.44*
Leaf 100 mg/kg	5.58 ± 0.16	6.44 ± 0.55	7.37 ± 0.45
Leaf 200 mg/kg	8.15 ± 0.56***	8.24 ± 0.46***	9.12 ± 0.25***
Stem 50 mg/kg	5.09 ± 0.34	6.06 ± 0.31	7.01 ± 0.63*
Stem 100 mg/kg	5.603 ± 0.23	6.00 ± 0.5^{3*}	6.07 ± 0.81
Stem 200 mg/kg	5.53 ± 0.68	5.74 ± 0.26	6.12 ± 0.56

Table 8: Analgesic activity of leaf and stem juice.

Statistical analysis: Two-way Annova followed by Dunnett's multiple comparison test, P value standard *vs.* leaf 200 mg/kg <0.05***, p value of overall data <0.018** (Table 9).

Doses	30 min	60 min	120 min
Leaf 50	29.62 ± 10.50*	23.60 ± 12.39	27.98 ± 5.82
Leaf 100	23.60 ± 12.39	20.54 ± 11.11	41.67 ± 8.61*

Leaf 200	20.54 ± 11.11***	69.31 ± 6.20***	81.25 ± 5.48***
Standard	69.31 ± 6.20	8.97 ± 2.42	19.35 ± 5.23
Stem 50	8.98 ± 2.42	24.62 ± 5.32	47.20 ± 12.46*
Stem 100	24.62 ± 5.33	41.32 ± 9.51*	27.64 ± 10.42
Stem 200	41.32 ± 9.50	31.49 ± 3.36	30.21 ± 7.98

Table 9: % Maximum possible analgesia.

Anti-inflammatory activity

Carrageenan induced paw edema: Male Swiss albino rats weighing 100 gm-150 gm were used. The animals were put on standard diet and water was provided ad libitum. The animals were fasted over night before the experimentation. The rats were divided into six groups (n=5). The anti-inflammatory activity of drug was assessed by the method described by Winter et al. Rats in group I were given normal saline and were treated as control. Rats in group II were administered diclofanac potassium in normal saline at the dose of 10 mg/kg body weight given intra peritoniallly and were kept as standard [15].

Rats in group III to group V were administered orally with the leaf juice and stem juice at the dose of 50 mg/kg, 100 mg/kg, 200 mg/kg body weight respectively. Since the LD 50 has not been determined during the acute toxicity study, the doses for this study where selected by trial and error method. The standard and drugs were given orally to the animal one hour prior to carrageenan injection. Acute paw edema was induced by injecting 0.1 ml of 1% (w/v) carrageenan solution, prepared in normal saline in sub-plantar region of the left hind paw of the rat. The perimeter of paw was measured by using vernier caliper. Measurements were taken at 0, 1, 2, 3 hours after the administration of the carrageenan.

Statistical analysis

Result are expressed as mean ± S.E.M. and difference in mean are determined by one-way ANOVA followed by post-hoc with Dunnets t-test; p values <0.05 were considered as statistically significant (Figures 5, 6 and Tables 10, 11).

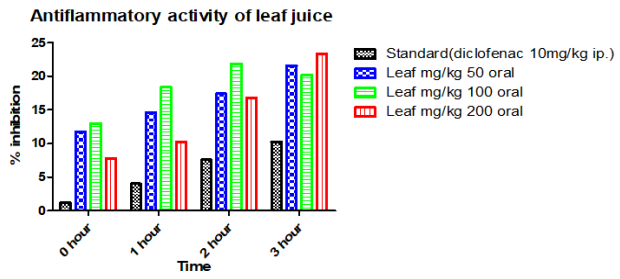

Figure 5: Anti-inflammatory activity of leaf juice.

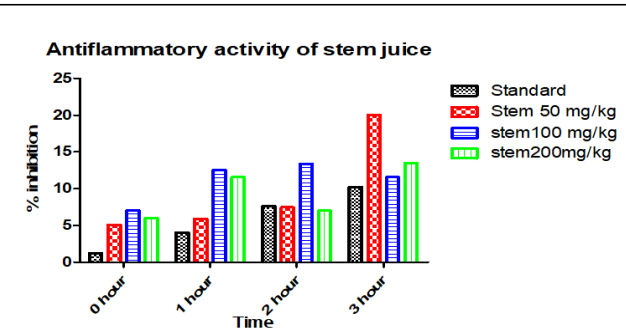

Figure 6: Anti-inflammatory activity of stem juice.

Groups	0 hr	1 hr	2 hr	3 hr
Control	5.77 ± 0.057	5.27 ± 0.057	5.012 ± 0.04	4.59 ± 0.06
Standard	5.70 ± 0.09	5.06 ± 0.08	4.63 ± 0.12	4.12 ± 0.1
Stem 50 mg/kg	5.48 ± 0.07	4.96 ± 0.14	4.64 ± 0.10	4.34 ± 0.07
Stem 100 mg/kg	5.62 ± 0.08	4.61 ± 0.05	4.34 ± 0.03	4.06 ± 0.05
Stem 200 mg/kg	5.43 ± 0.05	4.66 ± 0.05	4.36 ± 0.06	3.970.09
Leaf 50 mg/kg	5.09 ± 0.11*	4.49 ± 0.14*	4.14 ± 0.09*	3.67 ± 0.03*
Leaf 100 mg/kg	5.02 ± 0.28*	4.29 ± 0.12*	3.94 ± 0.10*	3.67 ± 0.05*
Leaf 200 mg/kg	5.35 ± 0.19*	4.70 ± 0.06*	4.17 ± 0.11*	3.51 ± 0.07*

Table 10: Anti-inflammatory activity of leaf and stem juice.

Groups	0 hr	1 hr	2 hr	3 hr
Standard	0.12	4.04	7.62	10.23
Stem 50 mg/kg	5.1	5.92	7.46	20.04
Stem 100 mg/kg	7.05	12.52	13.4	11.55
Stem 200 mg/kg	5.98	11.57	7.02	13.51
Leaf 50 mg/kg	11.76*	14.65*	17.40*	21.57*
Leaf 100 mg/kg	13.03*	18.44*	21.89*	20.26*
Leaf 200 mg/kg	7.80*	10.30*	16.80*	23.37*

Table 11: % Inhibition table.

Formulation studies

Mouthwash: Dentrifrices and mouthwashes which have only a cleansing refreshing and deodorizing action are defined as cosmetics. Those offered in gingivial or mucosal diseases or for the prevention of dental carries are drugs. However, many oral products contain therapeutic or antibacterial ingredients and also are intended to cleanse, refresh and deodorize. Within its narrowest definition water is the simplest mouth wash and next least complex type is aqueous saline. All mouthwashes are liquids usually in predominantly aqueous form or dilution just prior to use (Table 12).

Ingredients	Quantity	Use
Dry juice	1 g	Active drug
Menthol	0.05 g	Cooling agent
0il of cinnamon	0.13 ml	Flavour
Oil of clove	0.05 ml	Antibacterial
Formaldehyde solution	0.05 ml	Preservative
Sodium saccharine	0.04 g	Sweetener
Alcohol	4.5 g	Preservative
Water	Up to 100 ml	Vehicle

Table 12: Formulation No 3 (F3).

Procedure

Accurately weighed quantity of solid ingredients like dry juice menthol and sodium saccharine was taken in mortar and triturated well using pestle to form a uniform mixture. To this blend measured quantity alcohol was added. A small quantity of water was added and stirred the mixture properly. This mixture was transferred to measuring cylinder, the exact quantity of clove oil, cinnamon oil and formaldehyde solution was added and made the volume up to 100 ml using distilled water. Shaken well to form a uniform mixture. Transferred to a clean and air tight container.

A. arabica willd is an important plant of Indian medicinal flora, it is used as a chewing stick and is constituent of many herbal products seeds, leaf and stem are used for oral care.

Antimicrobial activity of babul stem and leaf dry juice

Antimicrobial activity of *Acacia nilotica* leaf juice and dried extract done by agar well diffusion method. Materials use for this nutrient agar, nutrient broth, Saubourd's Dextrose Agar (SDA), malt extract broth. Stains tested *Streptococcus fecalis*, *Staphylococcus aureus*, *Candida albicans* for *Streptococcus fecalis* blood agar was used, for *Candida albicans* 3 days incubated old culture was mixed with soft SDA and poured on hard solidified SDA. Then one bore was made with a cork bore in all the plates. One plate was kept as control for every organism. The plates of *Staphylococcus aureus* and *Streptococcus fecalis* were incubated at 37°C for 24 hours and *Candida albicans* for 3 days at 25°C.

The activity of dry juice was analysed, which indicated that there was no antimicrobial activity at 1% concentration in the selected strains, that same concentration uses in mouthwash formulation.

Patient perception study

This formulation was given to volunteers for patient perception response study using direct contact questionnaire method, the sample size was 13, formulation was tried for 15 days there was acceptance of taste by volunteers and also felt the formulation to be effective for health of gums and relief from respective disorders and compactness in gums, the magnitude of response was 85% for compactness. Patients had felt that this formulation do not stain the teeth. The maximum patient felt that it can be effective in bleeding gums,

sensitive gums and for other dental disorders. Volunteer were positive to use it as an oral healthcare product for regular use and not felt that colour can stain their teeth.

Discussion

Stem juice and leaf juice were obtained and the average % practical yields were 3.22 and 4.57 respectively. The juices when subjected to phytochemical tests were found to contain carbohydrates, tannins, steroids and flavonoids. Thin layer chromatography has been performed with benzene:methanol:acetone in 6:2:2 proportions as a solvent system showing two and three spots in stem juice and leaf juice respectively, when sprayed with vanillin sulphuric acid as a reagent. Spots are expected to be of tannins like catechin, protocateceuic acid and gallic acid.

Column chromatographic separation was tried on leaf juice and the separated white coloured compound was subjected to infra-red spectroscopy. Sample no. MRBA-41 was subjected to infra-red spectroscopy. The compound has given positive Molisch's test, on the basis of spectral data and chemical tests; MRBA-41 seems to be a complex hydrolysable tannin compound. However further structural elucidation needs the 1HMR, 13NMR and mass spectral data which could not be taken due to paucity of material. For determination of dose acute toxicity study was carried out in which no mortality was found up to the dose of 5000 mg/kg body weight and hence the doses of 50 mg/kg, 100 mg/kg, 200 mg/kg bodyweight were selected approximately for pharmacological studies.

In pharmacological studies an attempt has been made to analyze anti-inflammatory and analgesic activity and antimicrobial effect on selective strains. Leaf juice at the dose of 200 mg/kg body weight was found to be very effective in imparting analgesic effect. In the anti-inflammatory studies leaf juice at the doses of 50 mg/kg, 100 mg/kg and 200 mg/kg body weight was effective to reduce inflammation. The activity of leaf juice was more than that of stem juice but both can be claimed to have analgesic and anti-inflammatory activity. The activity may be due to presence of tannins, steroids and flavonoids.

The dry juice was incorporated into a mouthwash formulation at 1% leaf juice, 1% stem juice and 1% leaf and stem juice both of which formulation no. 3 with leaf and stem juice 1% both was better in taste, odour and colour. *A. arabica* Willd is an important tree of drier parts of India and has non replaceable place in rural parts. It is economically and ecologically both way important for the country.

Conclusion

Finding out new utilities through innovation and newer information through research will add value to this tree which ultimately will increase attention and spread of this tree which is also capable of reducing afforestration. Here we have tried to investigate juices of leaf and stem it is further possible to go for roots and specially juice of removed fresh bark which is known to contain huge amount of tannins and fresh flowers and aerial parts which are good source of steroids and flavonoids. There is need of suitable model for testing the gum tightening activity *in vitro*. The potential of both juices to combat local inflammation can be tried with other pharmacological models especially local inflammatory response can be analyzed.

References

1. Meena AK, Singh B, Niranjan U, Yadav AK, Nagaria AK, et al. (2011) A review on *Acacia nilotica* Linn. and it's ethnobotany, phytochemical and pharmacological profile. Res J Sci Technol 2: 67-71.
2. Dafallah AA, Al-Mustafa Z (1996) Investigation of the anti-inflammatory activity of *Acacia nilotica* and *Hibiscus sabdariffa*. Amer J Chinese Med 24: 263-269.
3. Khan TA, Tatke PA, Gabhe SY, Mahajan K, Tawde S, et al. (2011) Screening of methanol and water extracts of *Acacia nilotica* pods for *in vitro* anti-HIV activity. J Res Educ Indian Med 17: 89-94.
4. Jangade NM, Nagargoje PB, Shirote PJ (2014) Isolation, phytochemical and biological evaluation of *Acacia nilotica* (L) willd. leaf extract. Int J Pharmacog Phytochem Res 6: 179-182.
5. Chaubal R, Mujumdar AM, Puranik VG, Deshpande VH, Deshpande NR (2003) Isolation And X-Ray Study Of An Anti-Inflammatory Active Androstene Steroid From *Acacia nilotica.* Planta Medica 69: 287-288.
6. Verma PR, Deshpande SA, Rangari VD (2007) Antinociceptive activity of aqueous extract of *Pachyptera hymenaea* (DC.) in mice. J Ethnopharmacol 112: 203-206.
7. Winter CA, Risiey EA, Nuss GW (1962) Carageenan-induced edema in the hind paw of the rats as an assay for anti-inflammatory drugs. Expt Biol Med 111: 544-547.
8. Safari VZ, Kamau JK, Nthiga PM, Ngugi MP, Orinda G, et al. (2016) Antipyretic, antiinflammatory and antinociceptive activities of aqueous bark extract of *Acacia nilotica* (L.) Delile in albino mice. Pain Manag Med 2: 1-7.
9. Gazi MI (1991) The finding of antiplaque features in *Acacia arabica* type of chewing gum. J Clin Periodontol 18: 75-77.
10. Almas DR (2001) The antimicrobial effects of seven different types of Asian chewing sticks. Odontostomatol Trop 24: 17-20.
11. Angelo RU (2015) Efficacy of *Acacia nilotica* extracts towards microbicidal activity against pathogens. Int J Curr Microbiol Appl Sci 4: 33-42.
12. Adhikari B, Aryal B, Bhattarai BR (2021) A comprehensive review on the chemical composition and pharmacological activities of *Acacia catechu* (Lf) Willd. J Chem 2021: 1-11.
13. Anand U, Tudu CK, Nandy S, Sunita K, Tripathi V, et al. (2022) Ethnodermatological use of medicinal plants in India: From ayurvedic formulations to clinical perspectives-A review. J Ethnopharmacol 284: 114744.
14. Kumar M, Prakash S, Radha, Kumari N, Pundir A, et al. (2021) Beneficial role of antioxidant secondary metabolites from medicinal plants in maintaining oral health. Antioxidants 10: 1061.
15. Li RW, Myers SP, Leach DN, Lin GD, Leach G (2003) A cross-cultural study: Anti-inflammatory activity of Australian and Chinese plants. J Ethnopharmacol 85: 25-32.