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Prosopis juliflora (mesquite) gum exudate as a potential excipient

Sweta Basu[1], Majeti NV Prasad[1,2], Sateesh Suthari[1] and Boda Ravi Kiran[1]

Abstract

Gum exudate was obtained from *Prosopis juliflora* (Sw.) DC., which is abundantly available in north-west, central, west and south India. It was analysed for its phytochemical composition in aqueous extract and as well as by LCMS, GCMS, TGDTA and SEM to validate it's potential for use as an excipient (**Fig. 1**).

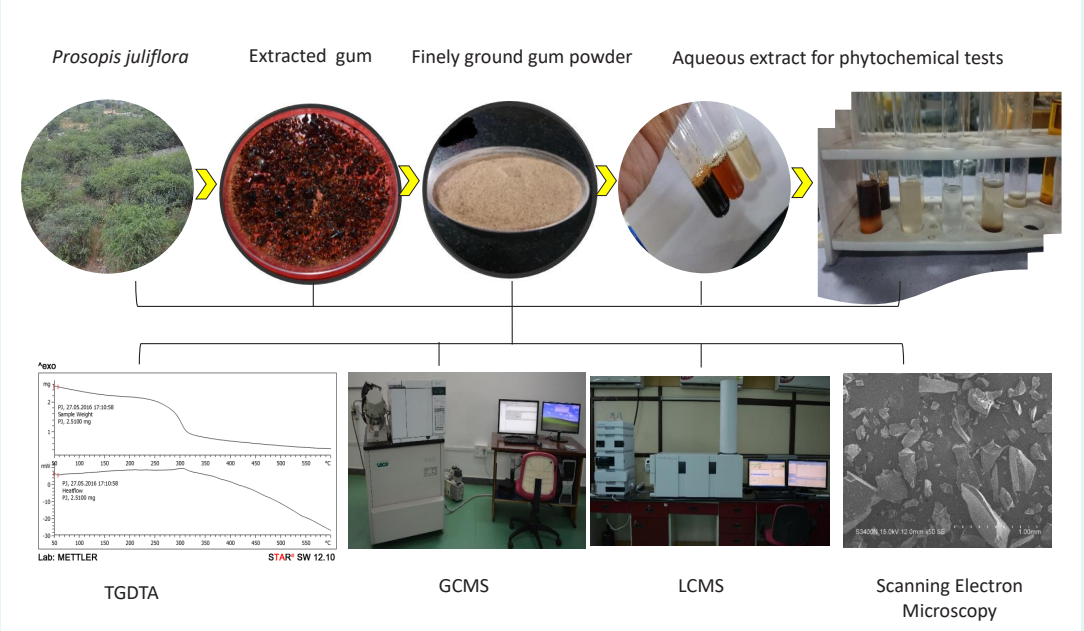

Figure 1. Graphic abstract of the analyses performed.

Introduction

Prosopis juliflora (Sw.) DC., an invasive weed in India has been highlighted for its bioremediation and bioeconomic potentials (1). *Prosopis juliflora* attracted considerable negative criticism from a section of plant scientists for being an alien invasive species. However, it provides a variety of resources and additional income to the local people through firewood collection, coal production etc. besides bestowing health care services as well (see the exhaustive bibliography cited in (1). Plant based excipients play a key role in the formulation of efficient drug delivery mechanisms (**Fig. 2**) (2). Selected examples of popular and better used plant excipient sources are as follows: *Aloe vera* (Xanthorrhoeaceae) as gelling agent, *Anacardium occidentale* (Anacardiaceae) as suspending agent, *Anogeissus latifolia* (Combretaceae) as binder, *Boswellia serrata* (Burseraceae) as natural suspending agent, *Buchanania axillaris* (Anacardiaceae) as substitute of gum Arabic, *Firmiana simplex* (Malvaceae) as emulsifying agent, *Senna tora* (Fabaceae) as binder and *Trigonella foenum-graecum* (Fabaceae) as gelling agent.

[1]Department of Plant Sciences, School of Life Sciences, University of Hyderabad Central University P.O., Gachibowli, Hyderabad, Telangana, India

[2]Present address: Visiting Professor School of Environment, Resources and Development (SERD), Room E120 Asian Institute of Technology (AIT) Klong Luang, Pathumthani, Thailand

Corresponding author: M.N.V. Prasad
E-mail: mnvsl@uohyd.ac.in

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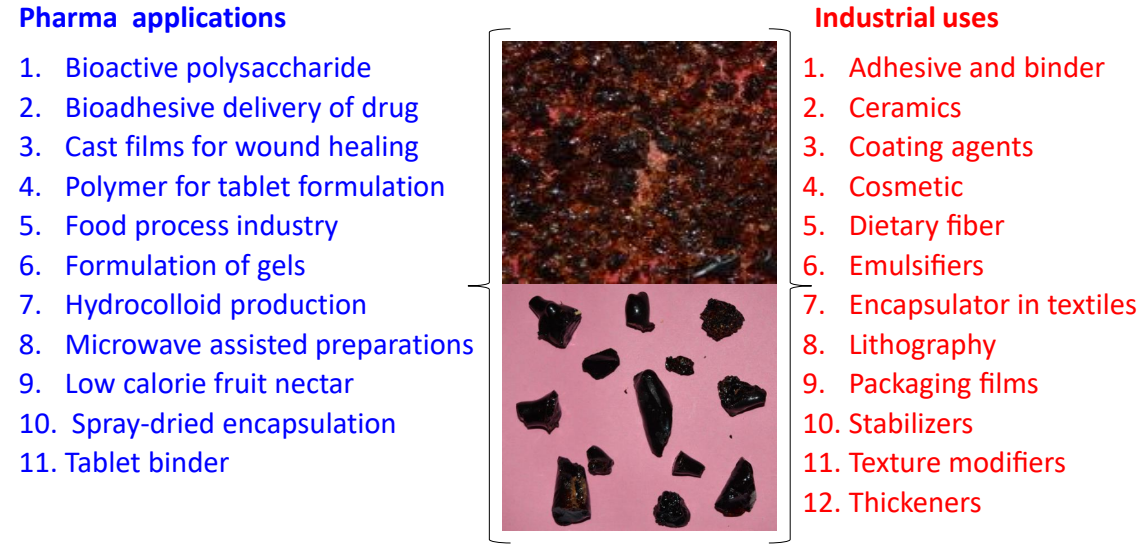

Figure 2. Economic importance of plant gums.

Over the years *P. juliflora* has spread far and wide to different parts of the world, beyond its native place, and even to India. Despite being an invasive species, mesquite plant has been known to have an array of interesting uses and can be used as a bioresource with immense potential (**Fig. 3**).

Figure 3. *Prosopis juliflora* habitat.

Some of it's known uses include: acts as natural herbicide on *Cynodon dactylon* [Bermuda grass] (3), it's alkaloid juliflorine is used to treat Alzheimer's disease (4), gum is used as encapsulation material for various food products (5), also applied for phytoremediation of sodic and degraded soil (6-8). Besides these, it has also been used as a very promising source of fuelwood, provide up to 30% feed stock in mini biomass based power plants and hence act as a viable bioenergy source (9-10). It is widely used for coal extraction, live fencing and to control erosion of the soil in Telangana particularly and India in general.

In view of these widespread uses of *Prosopis juliflora*, it has also been hypothesized that it's gum extract can be used as an excipient for effective drug delivery (11-12). Natural, plant gums are being exploited as vehicles for novel drug delivery(13). A natural excipient delivers bioactive substance efficiently, therefore is being preferred for drug formulations. Thus there is demand for inert and low cost excipients to serve as diluents, binders, disintegrants, adhesives, gallivants and sweeteners in the manufacture of tablets and capsules (14). To say that a certain polymer can be used as an excipient, it needs to undergo various quality checks and assessments (15). It includes both chemical and physical tests of the compound of interest. The complete development of a novel plant-based excipient involves an array of several rigorous tests, over a wide period of time.

The potential usage of gums, resins, gum-resins as excipients has opened up an exciting avenue in the field of plant based drug delivery system (13). A range of botanical excipients are sourced from plants such as *Aloe vera, Anacardium occidentale, Anogeissus latifolia, Boswellia serrata, Firmiana simplex, Gardenia latifolia, Leucaena leucocephala,* etc. (16). *Prosopis juliflora* gum exudate as excipient need to be explored due to its wide occurrence and immense range of utility as excipient.

Materials and Methods

The mesquite gum exudate was collected from Peddamaduru village of Jangaon district, Telangana and kept in air tight packs before they were further used to perform experiments (**Fig. 4**).

The gum crystals were then separated from any twigs, leaf/bark pieces and other foreign particles manually using sterilized forceps. The crystals were then finely grounded using a mortar and pestle and kept over night in a hot air oven at 180°C for proper drying. The fine powder was then checked for solubility in water and was then used for performing the various phytochemical tests. The powders were also used for performing other analytical tests like LCMS, GCMS and TGDTA (17).

pH characterisation

A 10% aqueous solution was made of the finely grounded *Prosopis juliflora* powder using double distilled water, and the pH

Figure 4. Gum exudate from *Prosopis juliflora* stem.

was checked using Digisun Electronics Digital pH Meter 7007.

Phytochemical evaluation
A wide range of chemicals were used to perform the tests on the *Prosopis juliflora* aqueous extract (18). All the chemical substances were of analytical grade from Sigma Aldrich Chemicals and used for all the tests.

Test for protein
A 10% aqueous extract was made by mixing the powder in double distilled water followed by vortex till the powder solubilised. Ninhydrin and Biuret Tests were performed (18).

Test for flavonoid
10% aqueous extract of the sample powder was made followed by vortex and ferric chloride and lead acetate tests were performed on it (18).

Test for carbohydrate
5% aqueous extract of the sample was used to perform the Benedict Test, Iodine Test and the Molisch Test (18).

Test for phytosterol
1g of the *Prosopis juliflora* powder was mixed with 2ml of glacial acetic acid followed by the addition of 3ml of acetic anhydride. This whole procedure was performed under a fume hood (18).

Test for fixed oils and fats
A 10% aqueous extract was made using the *Prosopis juliflora* powder. A few drops of the extract was put on a filter paper and was rubbed on with another piece of filter paper. A positive control was set up using immersion oil (18).

Test for alkaloid
2ml of 10% sample aqueous extract was treated with 0.2ml of 10% dil. HCl followed by the addition of 1ml Dragendroff Reagent. Also the sample extract was treated with 10% NaOH solution for the alkaline reagent (18).

Test for cardiac glycoside
2ml of the 10% aqueous extract of the sample was treated with 2ml of glacial acetic acid followed by 1 drop of 5% $FeCl_3$ solution and then 1ml of conc. H_2SO_4 was added along the sides of the test tube. This is the Keller-Killani test (18).

Test for steroid
To 2ml 10% sample aqueous extract 1ml of chloroform was added followed by the addition of 2 drops of conc.H_2SO_4 (18).

LC- Mass Spectrometry
For a further detailed compositional analysis the qualitative phytochemical tests are being supplemented with LC-MS data. The LC-MS analysis was done using MS Q-TOF and applying the Standard Metabolomics Method. A Dual ESI ion source and seven positive mass references were used.

Results and Discussion

pH analysis
Comparison of the common gums *Prosopis juliflora, Acacia nilotica* and *Firmiana simplex* indicated that *Prosopis* has the lowest pH and hence is most acidic amongst the three (**Fig. 5**). All these gums have been known to have a plethora of uses ranging from bioadhesives, neutraceuticals, pharmaceuticals, etc.

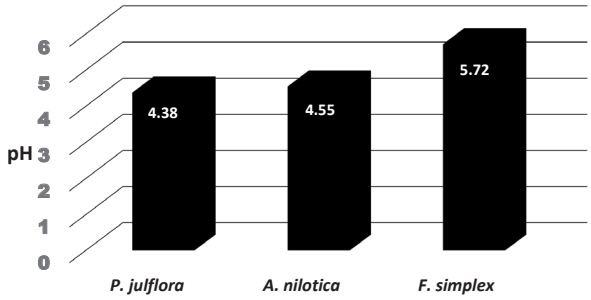

Figure 5. Comparison between the pH of 10% gum extracts of *Prosopis juliflora, Acacia nilotica* and *Firmiana simplex.*

Certain results have shown that *Prosopis* gum is highly bioadhesive and that increase in both ionic strength and pH favour bioadhesion (19). Moreover as it inherently shows a weak acidic nature it might be well suited for gastric drug delivery given that the stomach has a very low (acidic) pH range (20).

Phytochemical Tests
The phytochemical tests showed aclear presence of carbohydrate, flavonoid and cardiac glycosides in the *Prosopis juliflora* gum exudate. However, the tests performed are basic and preliminary in nature and requires the validation of other instrumental analysis (**Fig. 6**). The failure of experiment in some of the cases may be indicative of the presence of certain impurities alongside the sample powder or it may also imply that a water extract of the sample may not be suitable for the performance of such tests.

Biuret test (a)	Ninhydrin test (b)	Ferric chloride test (c)	Pb-acetate test (d)	Benedict's Test (e)	Iodine test (f)	Molisch test (g)	Phytosterol test (h)	Filter paper test (i)	Alkaline reagent test (j)	Dragendroff's test (k)	Keller- Killani Test (l)	Test for steroid (m)
—	—	+	NO	+	+	+	NO	—	NO	—	+	—

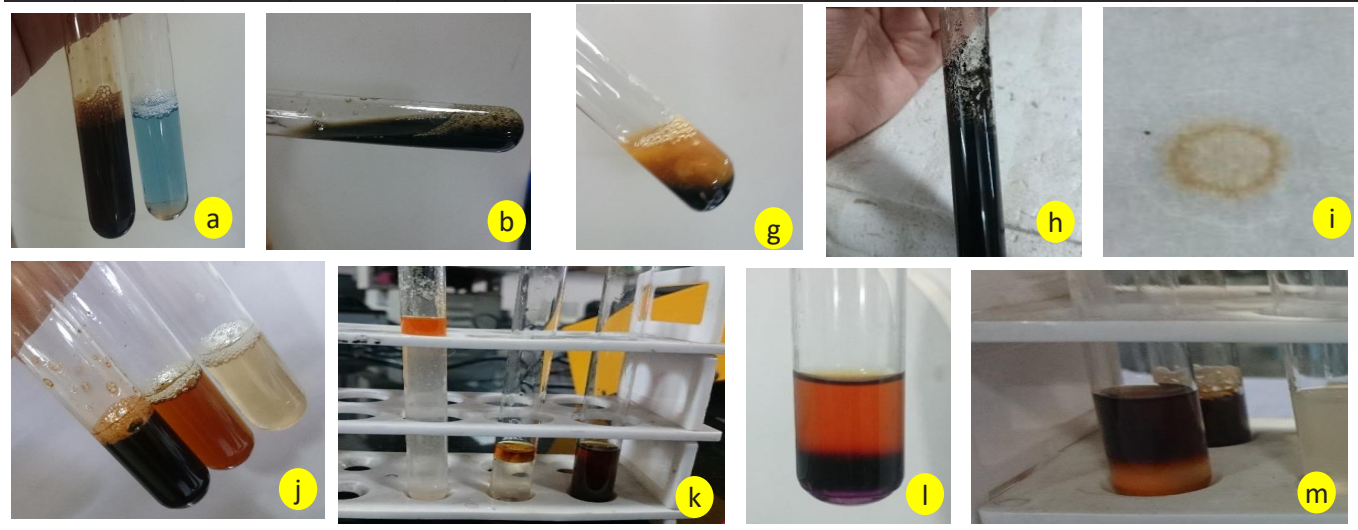

Figure 6. Tabulated form of the results of phytochemical test of *Prosopis juliflora* gum. The following symbols represent: (-) Negative result, (+) Positive result, (NO) Experimental failure.

Studies have already shown that mesquite gum has a series of arabinose-containing oligosaccharides (21) which tallies with the detection of carbohydrate in our sample. Cardiac-active pharmaceutical compositions contain a cardenolide glycoside (22) and the fact that our sample does contain cardiac glycoside gives it an added advantage of being used as an excipient. Pollen of *Prosopis* spp. is known to be a very rich source of flavonoids (23) and similarly it has been detected in our gum extract as well. The presence or absence of all these phytochemicals can be further confirmed by performing other quantitative tests given that the tests performed here are qualitative in nature.

TGDTA (Thermogravimetric Differential Thermal Analysis)

The thermodynamic qualities are very important to understand the dehydration and evaporation processes which are governed by water transition into crystalline or gaseous nature. The curves of TGDTA for mesquite gum depict at different proportions of water. The first stage was occurred after loss of water at set temperature (Tonset) at around 260°C, this loss can be attributed to typical polysaccharide content with 77.35%. The second stage was occurred with a residual content of 39.77% at end set temperature (Tendset) around 310°C, the residue proceeded towards the carbonization (**Fig. 7**).

SEM (Scanning Electron Microscopy)

Prosopis juliflora gum synthesized gold palladium sputter coated particles of different size (**Fig. 8**).

LCMS Analysis data

The ionization of the sample material was done both in positive and negative (**Fig. 9a,b**) charges have given two sets of peak data and values (**Table 1** and **2**). The masses obtained from the m/z ratios were compared to an online database using the software and certain compounds were identified from *Prosopis juliflora* sample.

Of the compounds detected luteolin, a flavonoid, is widely reported in natural products of plant origin. Flavones are reported to have a wide variety of pharmacological functions such as anti-oxidant, anti-inflammatory, anti-microbial and

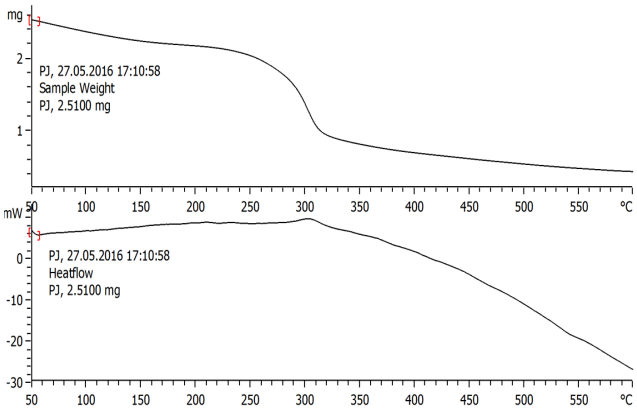

Figure 7. TGDTA thermogram of *Prosopis juliflora* gum with various amounts of water.

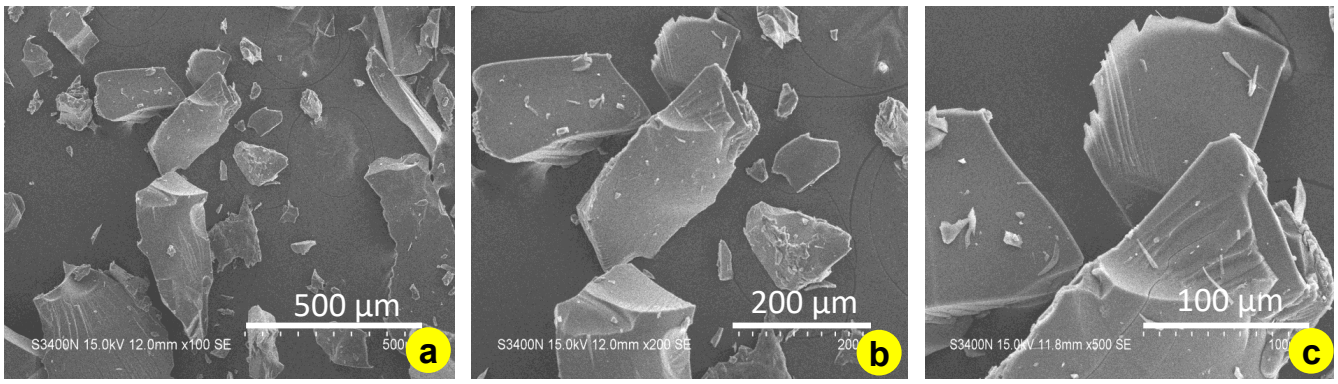

Figure 8. SEM micrographs of gold palladium sputter coated particles *Prosopis juliflora* gum.

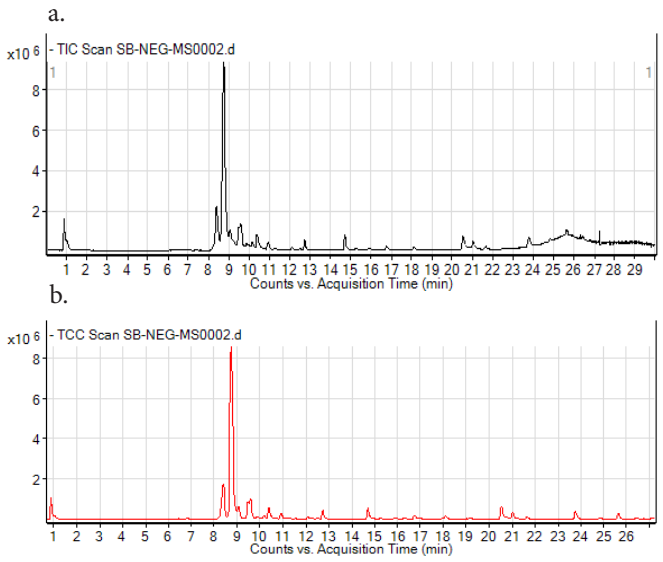

Figure 9a-b. Shows the peaks and the corresponding compounds detected in the negative mode of ionisation.

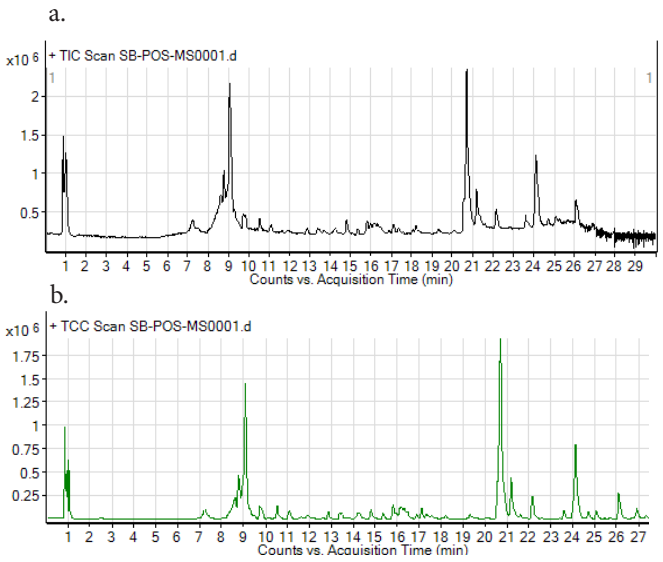

Figure 10a-b. Shows the peaks and the corresponding compounds detected in the positive mode of ionisation.

Table 1. Compounds detected in the negative mode

Compound Label	RT	Mass	Name	DB Formula	DB Diff (ppm)	Hits (DB)
Cpd 51: Luteolin	8.703	286.0477	Luteolin	$C_{15}H_{10}O_6$	-0.01	1
Cpd 97: Luteolin	10.041	286.0475	Luteolin	$C_{15}H_{10}O_6$	0.73	1
Cpd 107: Luteolin	10.431	286.0478	Luteolin	$C_{15}H_{10}O_6$	-0.3	1
Cpd 114: Isokaempferide	11.091	300.0629	Isokaempferide	$C_{16}H_{12}O_6$	1.8	1
Cpd 115: Luteolin	11.126	286.0481	Luteolin	$C_{15}H_{10}O_6$	-1.09	1
Cpd 119: Isokaempferide	11.3	300.0631	Isokaempferide	$C_{16}H_{12}O_6$	1.05	1
Cpd 127: Luteolin	12.304	286.0479	Luteolin	$C_{15}H_{10}O_6$	-0.68	1
Cpd 147: Prosopidione	15.869	208.1461	Prosopidione	$C_{13}H_{20}O_2$	1.25	1

Table 2. Compounds detected in the positive mode

Compound Label	RT	Mass	Name	DB Formula	DB Diff (ppm)	Hits (DB)
Cpd 127: Luteolin	10.219	286.0481	Luteolin	$C_{15}H_{10}O_6$	-1.08	1
Cpd 144: Prosopidione	13.473	208.1465	Prosopidione	$C_{13}H_{20}O_2$	-0.79	1
Cpd 187: Prosopidion3	16.879	208.1444	Prosopidione	$C_{13}H_{20}O_2$	9.19	1

anti-cancer. In animal model experiments, it has been established that luteolin inhibits angiogenesis, prevents carcinogenesis and reduce cytotoxic effects (24). Prosopidione, a monocyclic diketone, is an important constituent of *Prosopis juliflora* (25). Research has shown its anti-bacterial and anti-pyretic activities. Isokaempferide (flavonoid) is known to have anti-inflammatory function and experiments have confirmed its vital role as a bronchodilator (26).

Conclusion

Novel excipients from plant sources are an incredible step forward in the field of pharmaceuticals for new drug discovery for the welfare of living beings. Plant based materials are being used by people in various walks of life, hence making it imperative that they shall be introduced in medicines too. Not just active ingredients but usage of plant based natural excipients in medicines will be the infallible trend very soon. The fact that *Prosopis juliflora* is an abundantly available plant resource will further make it a stronger candidate as to why it's gum exudate is suitable as an excipient. Steps must be taken to ensure that the *Prosopis juliflora* gum passes all regulatory requirements for use in approved forms.

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Conflict of interest statement

The authors declare there is no conflict of interest.

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